4th International Conference on Infectious Diseases and Nanomedicine - 2023

December 15-18, 2023

Kathmandu, Nepal

ICIDN-2023

BOOK OF ABSTRACTS

Published by

ICIDN-2023 Secretariat
P. O. Box 24411, Kathmandu, Nepal
E-mail: icidn2023@gmail.com
Website: http://www.icidn-nepal.com

Venue

Nepal Academy of Science and Technology (NAST) Premise
Khumaltar, Lalitpur, Nepal

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4th International Conference on Infectious Diseases and Nanomedicine – 2023
(ICIDN – 2023)
December 15-18, 2023; Kathmandu, Nepal

WELCOME

Respected Distinguished Guests, Colleagues and Friends,

It is our great pleasure to welcome you in the 4th International Conference on Infectious Diseases and Nanomedicine (ICIDN-2023) from December 15 to 18, 2023 in Kathamndu, Nepal. The ICIDN-2023 is jointly organized by Nepalese Forum for Medical Microbiology (NFMM), Nepal Polymer Institute (NPI), CAS-TWAS Centre of Excellence for Biotechnology (CoEBio) at the Institute of Microbiology, Chinese Academy of Sciences (CAS) and the College of Biomedical Engineering and Applied Sciences (CBEAS) of Purbanchal University in Association with the Nepal Academy of Science and Technology (NAST) with support from The Company of Biologists, UK. The conference will be held on both in-person and online (virtual) platforms.

The conference will be attended by more than 120 participants, including renowned scientists from 7 countries across the globe. The previous editions of ICIDN held in Kathmandu (December 15-18, 2012, and 2015) and online (December 15-18, 2021) were highly successful in bringing together over 200 participants from more than twenty countries. The ICIDN-2023 focuses on molecular microbiology and epidemiology of infectious diseases and potential applications of nanotechnology for their diagnosis and treatments. Emerging infectious diseases, including COVID-19, antimicrobial resistance, drug design, and drug delivery will form an integral part of the ICIDN-2023 endeavors.

ICIDN-2023 will provide unique opportunity for presentation and sharing of innovations of microbiologists, immunologists, molecular biologists, epidemiologists, pathologists, chemists, pharmacists, polymer/biomedical engineers, material scientists, biotechnologists, nanotechnologists, clinicians, public health experts, and other biomedical scientists from both the academia and industries. The conference is hoped to be an important step to continue promoting interdisciplinary education and research in the field of microbiology, infectious diseases, material sciences and nanotechnology in Nepal and the region.

We extend our warm welcome to all our ICIDN-2023 delegates and wish for fruitful discussions and networking. We sincerely thank all our well-wishers and supporters, who have contributed to the ICIDN-2023 in various ways.

Sincerely,

Rameshwar Adhikari and Santosh Thapa
On behalf of the organizing committee of ICIDN 2023

PLENARY LECTURE

Plenary Lecture 1

Genome profiling of *Salmonella* Typhi clinical isolates from Northern India to identify resistance mechanism for development of novel drug candidates

Punit Kaur [1], Priyanka Sharma[1], Amit Katiyar[2], Arti Kapil[3]

[1]Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India
[2]Bioinformatics Facility. Central Core Research Facility, All India Institute of Medical Sciences, New Delhi, India
[3]Department of Microbiology, North DMC Medical College and HRH, Delhi, India

Background: Typhoid fever caused by *Salmonella* Typhi is an acute infection. Currently, development of anti-microbial drug resistance is continuously posing a major roadblock for the treatment of typhoid fever despite the introduction of newer antibiotics. This forms a key health problem in various endemic regions due to the emergence of multidrug resistant strains. Ceftriaxone has currently emerged as the drug of choice for fluoroquinolone resistant strains of *Salmonella* Typhi with azithromycin being used generally for uncomplicated cases of typhoid fever.

Methods: 133 clinical isolates from blood culture of typhoidal patients were analysed to predict antimicrobial resistance genes. DNA was extracted from each isolate and subjected to whole-genome sequencing (WGS) on the Illumina Mi-Seq platform. The possible genes were identified from the assembled sequences. The antimicrobial susceptibility of *S.* Typhi isolates against the 1st, 2nd and 3rd line-antibiotics were tested using the disc diffusion method and E-Test.

Results: WGS analysis revealed the presence of fluoroquinolone resistance conferring mutations in gyrA, gyrB, parC and parE genes of all strains. Acquired resistance determining mechanisms observed included catA1 genes for chloramphenicol resistance, dfrA7, dfrA15, sul1 and sul2 for trimethoprim-sulfamethoxazole and blaTEM-1B gene for amoxicillin. No resistance determinants were however, observed for the current drugs in use, ceftriaxone and cefixime.

Conclusions: A high genotype-phenotype correlation for chloramphenicol, ampicillin, co-trimoxazole, ciprofloxacin and ceftriaxone were observed in studied isolates of S. Typhi. The multiple genomes of Indian clinical strains also indicated that the bacterium was highly conserved. The isolates exhibited clonal nature with some diversity as the strains had acquired newer genes through Horizontal Gene Transfer. Furthermore, common mutational hotspots were explored to develop novel candidate molecules which could bypass this resistance mechanism.

KEYNOTE LECTURES

Keynote Lecture 1

Paradigm shift in medicine from treatment to prevention

Robert R. Bragg, PhD*; Samantha Mc Carlie; Wanja Swart

Department of Microbial, Biochemical and Food Biotechnology,
University of the Free State, Bloemfontein, South Africa
**Correspondence: Braggrr@ufs.ac.za*

There is growing concern on the increasing numbers of hospital acquired infections around the world. Most of the bacteria and yeast causing these infections are resistant to the commonly used treatment options, making the treatment of people who have contracted the pathogens very difficult.

There are limited options for treatment of bacterial infections in a post antibiotic era and the only really viable option is good biosecurity. In other words, kill the pathogens before they infect people. The best option for this is good quality disinfectants.

The concept of antibiotic resistance is very well known and studied. The concept of resistance to disinfectants is a less well-known concept and this can have very far-reaching consequences in the health care setting. Much research is currently underway to fully understand the concepts of disinfectant resistance. This includes studies on the genetics of resistance, how the genes are spread, and which genes are up or down regulated when the bacterium is exposed to a disinfectant.

Efflux pumps appear to be the main mechanism of disinfectant resistance. Many of the efflux pumps are active against various antimicrobials, including disinfectants and antibiotics. It has been demonstrated that antibiotic resistance in a bacterium can be produced even when the bacterium is not exposed to the antibiotic. If this bacterium develops resistance to a disinfectant, there can be an increase in antibiotic resistance.

An important concept is to establish if the bacteria isolated from your health care setting are inhibited by the disinfectants which you are using in your health care setting. If the bacteria develop resistance to a disinfectant, they can actually colonise the disinfectant bottles which has serious consequences to the health care facility.

Keynote Lecture 2

Structural and biochemical insights into the bacteriophage PlyGRCS endolysin targeting MRSA *Staphylococcus aureus* isolates and serendipitous discovery of its interaction with a cold shock protein C (CspC) from *E. coli*

Padmanabhan Balasundaram, PhD*

Department of Biophysics, National Institute of Mental Health and Neuro Sciences
(NIMHANS), Hosur Main Road, Bengaluru – 560029, India
**Correspondence:balapaddy@gmail.com, paddy@nimhans.ac.in*

Staphylococcus aureus is a pathogenic bacterium that causes infections ranging from skin abscesses to meningitis. Hospital-acquired S. aureus infections are particularly severe. Bacteriophage-encoded endolysins degrade the bacterial cell wall by hydrolyzing peptidoglycans, thus promising candidates to combat bacterial infections. PlyGRCS, a bacteriophage endolysin, contains a catalytic CHAP domain and cell-wall binding SH3_5 domain connected by a linker. It has been reported that though PlyGRCS has a single catalytic domain, PlyGRCS possesses both N-acetylmuramoyl-L-alanine amidase and D-alanyl-glycyl endopeptidase hydrolytic activities. Though the characterization of endolysins from different bacterial strains has been well documented, the literature has limited information about PlyGRCS. Here, we present the crystal structure of full-length PlyGRCS refined to 2.1 Å resolution for the first time. In addition, a serendipity finding revealed that PlyGRCS binds to the cold-shock protein C (CspC) of the expression host E. coli through interacting with the CHAP and SH3_5 domains. The lytic activity of PlyGRCS is reduced in the presence of CspC. The microbial assays of PlyGRCS against different strains of S. aureus and clinical isolates (MRSA, CA-MRSA, HA MRSA & LA-MRSA) showed substantial lytic activity against these clinical isolates. Overall, the crystal structure and biochemical results of PlyGRCS provide a molecular basis for the bacteriolytic activity of PlyGRCS against *S. aureus*.

Keynote Lecture 3

Dengue virus infection in Nepal: An increasing trend

Shiba Kumar Rai, PhD

Nepal Medical College, Attarkhel, Gokarneswor-8, Kathmandu, Nepal

Dengue virus infection, transmitted by Aedes aegypti (also by Ae. albopictus) mosquito, is an increasing health problem. The number of dengue cases has increased over eight-fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019 with deaths accounting in thousands. Currently, 3.9 billion people in 129 countries mainly in Asian countries are at the risk of dengue infection. Most of the new cases are reported from Brazil, Vietnam, Indonesia, the Philippines, Sri Lanka and others. First case of dengue was reported from Nepal in 2004 and in 2006 a small outbreak was reported with 32 cases throughout the country. In Nepal, dengue epidemic occurred in 2010, 2013, 2016, 2017, and 2019; of them 2019 epidemic was largest one with reported 17,992 cases. Last year (2022) the largest outbreak occurred with a total of 54,784 cases recorded from all 77 districts in 7 provinces. Of the 7 provinces Bagmati Province was hardest hit (77.4%) followed by Lumbini Province (9.2%), Koshi Province (4.2%), Gandaki Province (3.6%), Sudur Pashchim Province (2.5%), Madhesh Province (1.8%) and Karnali Province (1.2%). Most of the patients were aged 15-59 years and slightly more than half were males. Of the four serotypes existed in Nepal, DENV-1 DENV-2 and DENV-3 were common this year's outbreak (DENV-1 was predominant: 57.1%). This demands a precise mapping of dengue through integrated disease surveillance and evaluation of the dynamics of population level immunity on evidence-based policymaking in days to come.

Keywords: Dengue virus, emerging infection, increasing trends, Nepal

INVITED LECTURES

Invited Lecture 1

Emerging challenges in rabies elimination in Nepal

Sher Bahadur Pun, MD, PhD

Clinical Research Unit, Sukraraj Tropical & Infectious Disease Hospital,
Kathmandu, Nepal
Correspondence: drsherbdr@yahoo.com

Nepal has committed to end dog-mediated human rabies by 2030. However, the cases continue to rise in Nepal, despite the widespread availability of free vaccines at all government hospitals. So far, very little attention has been paid to understand the reasons behind the increase in rabies cases.

A total of 27 clinically confirmed dog-mediated rabies cases admitted to this hospital between August 2018 and December 2019 were included in this study. A one-on-one interview was conducted shortly after hospital admission. Of the total, 70% were under the age of 39 years. The median age of the patient was 28 years (range, 6-86); 78% were male. Majority of the cases (26%) believed that puppies do not carry rabies virus, which resulted in vaccine refusals. Other major factors associated with high vaccine hesitancy were due to incorrect counseling done by medicine sellers (22%), and minor bite wounds/scratches (19%) respectively. 11 % of the cases delayed or ignored to be vaccinated despite their family advice.

This study demonstrates lack of adequate knowledge about rabies, and the importance of vaccination, which were the major factors contributing to vaccine hesitancy, creating new challenges in elimination rabies disease in Nepal. Without addressing these emerging issues, dog-mediated rabies elimination program may not be readily achievable by the year 2030.

Invited Lecture 2

Environment surveillance of pathogenic viruses in the wastewater of Kathmandu, Nepal

Reshma Tuladhar, PhD[1]*, Samendra P Sherchan[2], Sarmila Tandukar[3], Bhawana Sharma[4], Bhushan Raj Shrestha[4], Jivan Shakya[1], Avilasha Sharma[1], Dinju Manandhar[1], Manita Aryal[1], Dev Raj Joshi[1]

[1]Central Department of Microbiology, Tribhuvan University, Nepal [2]Tulane University, New Orleans, LA, USA [3]Policy Research Institute, Kathmandu, Nepal [4]Environment and Public Health Organization, Kathmandu, Nepal

**Correspondence: reshma.tuladhar@cdmi.tu.edu.np*

Background: Wastewater based surveillance has gained global attention for community detection and investigation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This study aims to determine the feasibility of wastewater-based epidemiology as a proxy method for monitoring different viruses in Kathmandu Nepal.

Methods: We used Skimmed milk flocculation method for the concentration of viral RNA from sewage samples collected from the three key hot spots (vulnerable zones) during pandemic and river water samples from five catchments post-pandemic. Viral RNA was detected and quantitated by real-time qPCR. The spike gene was sequenced with NGS strategy using the Illumina MiSeq only from SARS-CoV-2 virus detected during pandemic. Variants were assigned in the sequences using "kallisto" bioinformatics pipeline. Similarly, we detected Hepatitis A virus, Enterovirus and Pepper mild mottle virus (PMMoV) from post-pandemic samples.

Results: The most frequent mutation detected was SNP - T477K followed by D614G and L452R. These mutants are attributed to the stronger affinity of the spike protein for the ACE-2 receptor making it more transmissible and infectious. Among the assigned eleven variants, Delta was the dominant variant until mid-December 2021, which was later replaced by Omicron and correlated with clinical surveillance data. Nepal had Omicron wave with massive positivity rates in January 2022. The post-pandemic samples were positive for SARS-CoV-2 concurrent to the rise in clinical cases during the month of April in 2023. Similarly, Hepatitis-A virus and Enterovirus were detected from the downstream river catchments of densely populated areas.

Conclusions: The results highlight that surveillance of pathogenic viruses in wastewater is feasible and can be utilized as supplementary to clinical surveillance for effective public health interventions. Thus, makes it a potential surveillance intervention for early warning system.

Key words: Enterovirus, Hepatitis A virus, SARS-CoV-2, Skimmed milk flocculation.

Invited Lecture 3

Merger of ayurveda and biotechnology for study of anti-cancer activity of medicinal plants of Nepal

Basanta Lamichhane, Pritish Shrestha, Sandeep Adhikari, Jaya Bhandari, Bhupal Govinda Shrestha, PhD

Department of Biotechnology, Kathmandu University, Bakhundole, Dhulikhel, Nepal

Background: In the last twenty-five years, approximately half of all newly registered chemotherapeutics have been developed from natural products isolated from plants, fungi or microorganisms. Nepal because of her geographical biodiversity, is home to flora and fauna of tropical, sub-tropical and alpine origin. These herbs have been used since ancient times to treat numerous diseases.

Methods: In the current research, medicinal plants were extracted using methanol. The dried extract was dissolved in DMSO and used to perform different assays, like phytochemical presence, antioxidant and antimicrobial activity and anticancer activity using cancer cell lines and western blotting.

Results: Phytochemical screening of extracts revealed presence of various phytochemicals like alkaloids, flavonoids, terpenoids, coumarin, saponin, reducing sugar, glycosides, tannin, and steroid. The plant extract also showed antioxidant assay namely DPPH (Diphenyl-2-picrylhydrazyl) free radical scavenging activity. Antimicrobial screening showed sensitivity against *Candida albicans*, *Salmonella* Typhi. *Pseudomonas aeruginosa*, *Klebsiella pneumonia*e, *Staphylococcus aureus*, and *E. coli* by the plants. We also looked at anti-cancer activity in human normal and cancer cell lines and we saw cytotoxic effect of the extracts against cancer cells lines as seen by MTT assay and CV staining and looked at their target proteins through Western Blotting.

Conclusions: Medicinal plants of Nepal possess different biochemical activity including anti-cancer activity. Anti-cancer activity of the plant is through upregulation of p53 protein.

Invited Lecture 4

Morphology, Deformation, and Micromechanical Behavior of Electrospun PCL/PLLA/Gelatin/Vitamin D3 and Nano-hydroxyapatite-Blended Composite Nanofibrous Scaffolds

Komal Prasad Malla[1, 2 ,3], Sven Henning[4, 6], Ralf Lach[5, 6], Herbert Jennissen[7], Goerg Michler[3, 6], Mario Beiner[3,4], Rameshwar Adhikari[1]*

[1]Tribhuvan University, Central Department of Chemistry, Kathmandu, Nepal
[2]Pokhara University, Faculty of Health Sciences, Pokhara, Nepal
*[3]Martin-Luther-University Halle-Wittenberg, Institute of Physics, Von-Danckelmann-Platz 3, 06120 Halle Germany [4]Fraunhofer Institute for Microstructure of Materials and Systems (IMWS), Walter-Hülse-Straße 1, 06120 Halle / Saale, Germany [5]Polymer Service GmbH Merseburg (PSM), Geusaer Straße 81f, 06217 Merseburg, Germany [6]Institute for Polymer Materials e.V. (IPW), Eberhard-Leibnitz-Straße 2, 06217 Merseburg, Germany [7]University of Duisburg-Essen, Department of Biochemical Endocrinology, Institute for Physiological Chemistry, Hufeland Strasse 55, 45122 Essen, Germany *Correspondence: nepalpolymer@yahoo.com*

Background: Nano-hydroxyapatite (nano-HAp) is a bioceramic widely used as filler in various polymeric fibrous scaffolds for bone tissue engineering which may change the morphology and micromechanical behavior of the polymeric fibers and improve their biocompatibility.

Methods: In this study, different volume fraction percentages of biogenic nano-HAp and vitamin D3 were incorporated into electrospun biodegradable polymer blend fibrous scaffolds comprising polycaprolactone (PCL), poly-L-lactic acid (PLLA), and gelatin. The chemical, morphological, and micromechanical properties of the scaffolds were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) as well as transmission electron microscopy (TEM) and tensile testing.

Results: The FTIR analysis shows the presence of functional groups of fillers and polymers and their molecular interactions on the scaffold's fibre. The microscopic results reveal the effect of fillers on the fibre morphology (including diameter, phase separation and texture of the fibres) and response of the fibers under tensile deformation. It has been found that 3 % by volume of nano-HAp suspension induced the maximum fibre diameter of 4.3 µm and the addition of 6 % to 12 % filler led to an increase in a tensile strength from 0.7 MPa to 5.6 MPa and strain at break from 2 % to 37 % for the composite fibers compared to the neat fibers.

Conclusions: The observed improvement in mechanical behavior can be correlated to a transition from crazing (in the neat fibres) to thin layer yielding in the composite fibers. Such scaffolds might show good results of *in vitro* differentiation and proliferation of bone cells and can be applied in bone tissue engineering.

Keywords: nano-hydroxyapatite, biodegradable polymers, fibrous scaffolds, electrospun, characterization, bone tissue engineering.

Invited Lecture 5

Challenges in visceral leishmaniasis elimination in Nepal

Kishor Pandey, PhD

Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
Correspondence: kishor.pandey@cdz.tu.edu.np

Background: Visceral leishmaniasis (VL) also known as kala-azar is a neglected tropical disease that is becoming more prevalent and is linked to poverty. In Nepal, VL infections have been spreading into new regions even after the elimination campaign was launched in 2005. This study aims to investigate VL challenges in Nepal.
Methods: In the present study, a retrospective analysis of 40 years of data from the Epidemiology and Diseases Control Division Similarly, other relevant information was obtained from annual health reports and published papers.
Results: From 1980 to 2019, a total of 34 564 cases and 584 fatalities related to VL were documented. Up to 2006, there was a consistent rise in VL, and after that number of cases and deaths gradually decreased. The greatest recorded number of deaths (65 deaths) occurred in 1995, whereas the most recorded number of VL cases (2229 cases) occurred in 2003. The main challenges of VL elimination are the expansion of VL in new geographic areas, lack of knowledge about VL among the general population, proper diagnosis and treatment, no regular surveillance of sandflies, not enough data of vector, reservoir, incubation, and severity of VL, asymptomatic VL cases and post-kala-azar dermal leishmaniasis.
Conclusion: Overall, tackling these challenges requires a multi-faceted approach involving collaboration between the government, healthcare professionals, researchers, and the community. By raising awareness, improving healthcare infrastructure, and addressing socio-economic factors, Nepal can make significant progress in eliminating VL by strengthening surveillance and early detection, vector control measures, community engagement and education, accessible healthcare services, integration with existing healthcare programs, collaboration, and research.
Keywords: Visceral leishmaniasis, public health, vector control, epidemiology

Invited Lecture 6

Temporal analysis of SARS-CoV-2 variants during the COVID-19 pandemic in Nepal

Samrat Paudel[1], Amol Dahal[1], Hitesh Kumar Bhattarai, PhD[1]*

[1]*Department of Biotechnology, Kathmandu University, Dhulikhel, Nepal*
**Correspondence: hitesh.bhattarai@ku.edu.np*

Background: Before the omicron variant, Nepal suffered two waves of SARS-CoV-2 infections, one in the year 2020 and another in the year 2021. Although the government of Nepal keeps a detailed record of daily coronavirus infections and deaths throughout the country, and publishes the result every day, genomic surveillance of mutants in the country has lagged behind. Sequencing of COVID-19 samples has been conducted sporadically during the pandemic.

Methods: From the GISAID database, 127 high-quality sequences deposited by different health authorities in Nepal were collected and analyzed before the arrival of o-micron variant.

Results: From the analysis, it can be concluded that at least two variants of concern, alpha and delta, and one variant of interest, kappa, were detected in Nepal in 2021. As in other countries, the delta variant outcompeted the kappa and alpha variants and by July 2021 had established itself as the dominant variant. It can be hypothesized that the second wave in Nepal was primarily caused by the delta variant. Further, phylogenetic tree analysis suggests cases of local transmission and global transmission of coronavirus.

Conclusion: This analysis reveals the global nature of the disease, where variants arising in one part of the world can quickly spread to other parts of the world and can also spread through individual communities.

Keywords: COVID-19, Nepal, variants, delta, temporal, analysis, phylogenetic tree

Invited Lecture 7

Colistin-resistant *mcr-1* gene in *E. coli* from poultry and slaughterhouse wastewater

Pramila Parajuli*, Pratikshya Pokharel, Bunu Khanal, Anisha Shrestha, Shyam Prasad Pant
St. Xavier's College, Maitighar, Kathmandu, Nepal
**Correspondence: parajuli.p@sxc.edu.np*

Background: The Emergence, Spread, and persistence of colistin resistance in bacteria and diseases caused by such bacteria is a pressing challenge. Antimicrobial resistance poses a serious and growing risk to public health, necessitating a response on a global scale to create strategies that work and reduce the emergence and spread of this phenomenon in clinical and environmental settings. To confront the challenges of antimicrobial resistance, WHO has authorized an arrangement of activity plans including increasing awareness of the magnitude and effect of antimicrobial resistance through reconnaissance, research, education, training, and communication.

Methods: A total of 763 colistin-resistant *E. coli* isolates were isolated from a cloacal swab of poultry and slaughterhouse wastewater and identified based on cultural characteristics, Gram's staining, and biochemical tests. MIC of the isolates against colistin was determined by broth dilution method and then molecular analysis was conducted to detect the presence of *mcr*-1 gene by conventional PCR method.

Results: A total of 71% *E. coli* isolates from poultry cloacal swabs and 62.4% *E. coli* isolates from Slaughterhouse wastewater were resistant to the last line drug, colistin. The Minimum Inhibitory Concentration (MIC) of the isolated *E. coli* against colistin was determined by broth dilution and the MIC value ranged from 2µg/ml to more than 64µg/ml. Higher MIC value was observed in the isolates from poultry than from slaughterhouse wastewater. A total of 100 colistin-resistant *E. coli* from Poultry cloacal swabs and 53 isolates from slaughterhouse wastewater were selected for molecular analysis to detect the *mcr*-1 gene responsible for colistin resistance. A total of 22% of colistin-resistant isolates from poultry showed the presence of the *mcr*-1 gene while 15% of colistin-resistant *E. coli* isolates from slaughterhouses harbored the *mcr*-1 gene.

Conclusion: A high number of colistin-resistant organisms were present in poultry and in the environment. Thus, awareness programs and strict policies should be implemented to reduce the risk of exposure to resistant organisms to protect human and environmental health.

Keywords: Colistin Resistance, wastewater, cloacal swab, MIC, *mcr* gene

Invited Lecture 8

Circulation of Antibiotic-resistant Bacteria with Their Resistance Determinants in the Water Environment of Kathmandu, Nepal

Dev Raj Joshi[1], *Sudeep K C*[1,2], *Santosh Khanal*[1,2], *Tista Prasai Joshi*[2]

[1]Central Department of Microbiology, Tribhuvan University, Nepal
[2]Nepal Academy of Science and Technology, Nepal
E-mail: dev.joshi@cdmi.tu.edu.np

Abstract

Environmental antibiotic resistome is the pool of antibiotic resistant bacteria and their determinant genes including mobile genetic elements. The environmental resistome pose significant risk of transmission and circulation antibiotic resistance in community and clinical settings. The understanding on circulation of antibiotic resistant bacteria carrying determining genes and mobile genetic elements and monitoring the sources in wastewater and drinking water environment is important public health strategy to mitigate global burden of antibiotic resistance. In this talk, we present the occurrence and circulation of β-Lactam and other antibiotic resistance bacteria and their genes including the factors associated with horizontal gene transfer like integrons. We report present of Extended-Spectrum β-Lactamase (ESBL) and carbapenemase producing bacteria and their genes in respective β-Lactam resistant bacterial isolates recovered from wastewater samples from hospital, pharmaceutical industries, municipal sewage and a receiving river in Kathmandu, Nepal. We correlate the increased frequency of antibiotic resistance with increased concentration of organic matter indicating the role of organic pollutants in the induction and spread of resistance in river water. We found high-frequency ESBL and Carbapenemase-producing phenotype and genotype of *E. coli* and *Klebsiella* spp in hospital and municipal wastewaters and receiving rivers, indicating antibiotic resistance in river was impacted by these sources. We also found resistant bacteria and genes in groundwater. We assume that municipal sewage and hospital wastewater are key reservoir of antibiotic resistome whereby dissemination to surface water and groundwater and drinking water. This implies a need for monitoring and control strategies to prevent the circulation of resistance in the environment and its potential consequences on human health.

Invited Lecture 9

Geometrical and Thermodynamical Stability of Adducts of Selected Phytochemicals with Helicase Protein of nCoV-19

Jhashanath Adhikari Subin[1, *], Rameshwar Adhikari[2, 3]

[1]Bioinformatics and Cheminformatics Division, SRTN, Kaushaltar, Bhaktapur 44800
[2]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu 44600
[3]Research Centre for Applied Science and Technology, Tribhuvan University, Kirtipur, Kathmandu 44600

*Corresponding email: subinadhikari2018@gmail.com

Abstract

Background: The stability of the protein-ligand adduct is of utmost importance in assessing the effectiveness of inhibition of the normal functioning of the protein by the ligand docked to it. The non-covalent interactions that are present between the key amino acid residues and the ligand molecule determine the strength by which the ligand is bound to the protein at its orthosteric site. The stability of the protein-ligand complex evaluated by various geometrical parameters extracted from the molecular dynamics trajectory helps to provide a hit like candidate. The thermodynamical parameter, binding free energy change, predicts the spontaneity of the complex formation reaction and provides additional support to the stabilty assessment. Some phytochemicals from plants with ethnobotanical values were studied by computational methods for assessing the geometrical and thermodynamical stability of the adduct formed with the helicase protein of nCoV-19.

Methods: Molecular docking calculations were performed to find a ligand with the strongest interactions with the protein. The adducts with the best docked scores were subjected to 200 ns long molecular dynamics similations to determine the geometrical stability. The MMPBSA method was applied to the equilibrated part of the trajectory to calculate the binding free energy changes.

Results: Some of the molecules were proposed as the top candidates based on docking scores. These phytochemicals possessed better values than those calculated for the selected drugs and a native ligand. The stability of the complex was inferred from RMSD, RMSF, Rg, SASA, and hydrogen bond count. The images at multiple times showed that PTC01 nearly retained the docked position with moderately smooth nature of the RMSD curve. ΔG_{BFE} hinted at the spontaneous nature of protein-PTC01 adduct formation and its frame-by-frame analysis complemented the geometrical results.

Conclusions: Helicase-PTC01 adduct possessed the best geometrical and thermodynamical stability and PTC01 could be proposed for further experimental characterization.

ORAL PRESENTATIONS

Oral Presentation 1

MiR155HG: A double-edged sword in promoting antiviral innate immunity

Kul Raj Rai, PhD[1,2*], Yuan Liao[1], Mengjuan Cai[1], Haori Qiu[1], Faxin Wen[1], Min Peng[1], Song Wang[1], Shasha Liu[1], Guijie Guo[1], Xiaojuan Chi[1], Mohamed Maarouf[1], Yuhai Chen[1], Shile Huang[1] and Ji-Long Chen[1*]

[1]Key Laboratory of Animal Pathogen Infection and Immunology of Fujian Province, College of Animal Sciences, Fujian Agriculture and Forestry University, Fuzhou, China
*[2]Department of Microbiology, Shi-Gan International College of Science and Technology, Kathmandu, Nepal *Correspondence: kulrajrai701@gmail.com*

Background: MiR155HG, encodes both microRNA-155 (miRNA-155) and a long noncoding RNA (lncRNA-155). To understand the distinct functions of miRNA-155 and lncRNA-155, we created miRNA-155 knockout (KO) mice lacking only 19 bp of the miRNA-155 core sequence, while leaving lncRNA-155 intact.

Methods: The CRISPR/Cas9 technique was employed to generate miRNA-155KO mice. For studying the impact of miRNA-155 and its corresponding lncRNA-155 on antiviral immune responses, three groups of mice were used: miRNA-155KO, lncRNA-155KO, and wild-type mice. Additionally, various human and mouse cells were utilized in the investigation. The study focused on the immune responses triggered by the infection of RNA and DNA viruses. Techniques such as RT-PCR, qPCR, Western Blot, among others, were employed to analyze and assess the effects at a molecular level.

Results: Previously generated lncRNA-155KO mice were more susceptible to influenza virus (RNA) and pseudorabies virus (DNA) infections compared to miRNA-155KO mice, characterized by lower survival rates, higher body weight loss, and a higher viral load. We found that miRNA-155-5p enhances antiviral responses by positively regulating the activation of signal transducer and activator of transcription 1 (STAT1). However, virus infection induced activity of STAT1 varied greatly among the different mice groups (wild type > miRNA-155KO > lncRNA-155KO). Similarly, the expression levels of several critical interferon-stimulated genes (ISGs) differed significantly among the groups. It was found that lncRNA-155 promoted the production of interferon-beta (IFN-β) during viral infection, whereas miRNA-155 showed no significant effect on virus-induced IFN-β expression. Additionally, we observed that lncRNA-155 loss in mice dramatically inhibited the virus-induced activation of interferon regulatory factor-3 compared to both miRNA-155KO and wild-type animals. Furthermore, we found that lncRNA-155 still significantly suppressed viral infections even when the miRNA-155 derived from lncRNA-155 was deleted or blocked.

Conclusions: These findings demonstrate that both lncRNA-155 and miRNA-155 accelerate antiviral responses through distinct mechanisms, suggesting MiR155HG acts as a double-edged sword in promoting antiviral innate immunity.

Oral Presentation 2

The hormetic effect of disinfectants: Making pathogens stronger

Samantha J Mc Carlie, Robert R. Bragg

Department of Microbiology and Biochemistry, University of the Free State,
Bloemfontein, South Africa

Hormesis or the hormetic effect, in terms of microorganisms, is a phenomenon that occurs when an antimicrobial used at a low concentration or dose has a stimulatory effect on microbial growth instead of the intended inhibitory or microbicidal effect. This has been observed for several antibiotics such as tobramycin, tetracycline, and norfloxacin in ESKAPE bacterial pathogens such as *Pseudomonas aeruginosa*. Recently research has shifted on to whether this phenomenon can be elicited by bacteria challenged by antiseptics, sanitisers and disinfectants. An increasing number of hospital-acquired infections have been attributable to *Serratia sp*. Therefore, the effect of sub-lethal levels of disinfectants on the growth *Serratia sp. HRI* (resistant isolate) and *Serratia marcescens* ATCC 13880 (type strain) were evaluated. The bacteria were cultivated in sub-lethal levels of the following antimicrobial compounds: benzalkonium chloride (BAC), Didecyldimethylammonium chloride (DDAC) and VirukillTM. The maximum specific growth rate, doubling time and cell counts were compared between the two bacteria for all three QAC-based disinfectants. The results revealed significant increases in maximum specific growth rate and shorter doubling times for the resistant isolate, Serratia sp. HRI, when cultivated in sub-lethal levels of BAC and DDAC antimicrobials. These results confirm that the hormetic effect is not unique to antibiotics but can be conferred by antiseptic, disinfectant and sanitiser products as well. This is deeply troubling as these compounds are vitally important in wound care, and instrument and equipment cleaning in healthcare. Incorrect dosage or inaccurate dilution of these products can cause an inadvertent stimulation of microbial growth instead of inhibition. The significant stimulatory effect for *Serratia sp. HRI* presented here represents the first time that hormesis has been observed in a Gram-negative bacterium for any disinfectant.

Oral Presentation 3

Antimalarial efficacy of methanolic mangrove extract against *Plasmodium falciparum – In vitro & In vivo*

M. Syed Ali, PhD

*PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Chennai-600119, Tamilnadu, India *Correspondence: syedmicro555@gmail.com*

Malaria is one of the most important life-threatening infectious diseases in the tropics. Despite the effectiveness of artemisinin-based combination therapy, reports on reduced sensitivity of the parasite to artemisinin in India warrants screening for new potential antimalarial drugs for future use. The newest antiplasmodial drug from plants is needed to overcome this problem. Numerous mangroves and mangal associates are used as folklore medicine to treat various human diseases. The mangrove plant species are a good source of potential bioactive entities which exhibit many therapeutic properties. The present study was carried out to test the antiplasmodial activity of five mangrove plant species distributed along the Southeast coast of India. *Rhizophora mucronata* mangrove plant extracts exhibited *in vitro* and *in vivo* antiplasmodial activity against chloroquine-sensitive *Plasmodium falciparum*. The extract of *R. mucronata* exhibited high antiplasmodial activity and *in vivo* activity. Statistical analysis reveals that, significant antiplasmodial activity ($P<0.05$) was observed between the concentrations and time of exposure. The chemical injury to erythrocytes was also carried out and it shows that no morphological differences in erythrocytes by the extract of mangrove plants after 48 h of incubation. The screening for phytochemical constituents in the mangrove plants were carried out and it reveals that, the presence of alkaloids, triterpenes, flavonoids, tannins, catachin, anthroquinone, phenols, sugars, and proteins and antioxidant activity and hematological carried out. This study shows that the mangrove plants had a source of lead compounds for the development of new drugs for the treatment of malaria.

Oral Presentation 4

Antioxidant and radical scavenging potential of silver nanoparticles catalyesd by bio constituents in *Terminalia arjuna* bark and *Smilax glabra* Roxb

V. Anuradha, PhD[1]*, M. Syed Ali[2]

[1]PG & Research Department of Biochemistry, Mohamed Sathak College of Arts & Science, Chennai-600119, Tamilnadu, India
[2]PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Chennai-600119, Tamilnadu, India
**Correspondence*: *vanuradha2712@gmail.com*

Biosynthesized silver nanoparticles are widely used in pharmacy and medicine. In particular, AgNPs synthesized and mediated by plant extracts have been shown to possess several biological activities. In the present study, AgNPs were synthesized using Terminalia arjuna bark and Smilax glabra extract as reducing agent. The synthesis of AgNPs was confirmed by visual observation as a change in color of the solution impregnated with silver. The morphology of the AgNPs, average size, and presence of elemental silver were characterized by UV-Visible spectroscopy, scanning electron microscopy, and FT-IR. In vitro antioxidant activity such as Total antioxidant, DPPH, Ferric reducing antioxidant assay and ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging assay were carried out. To test the anti-inflammatory activity of the extract, Inhibition of albumin denaturation and HRBC Membrane Stabilization Method was done as per standard method. The total antioxidant activities of standard the silver nanoparticles were measured in the concentration of 100 to 500 µg/ml and the OD values were noted. As part of the investigation into the mechanism of the anti-inflammation activity. The IC50 value of silver nanoparticles of *Terminalia Arjuna* and *Smilax glabra Roxb* was observed (398.12µg/ml & 402.58 µg/ml). They can be used to address a number of challenges in the field of nanomedicine. But it must be remembered that they can also possibly cause adverse biological effects at the cellular and subcellular levels.

Oral Presentation 5

Antibiotic resistant bacteria detected in pigeons' droppings in selected temples of Kathmandu: Urgent need to combat antimicrobial resistance

Sofia Shahi Thakuri[1], Gagan Gurung[1], Sanjib Adhikari[1],
Jyoti Acharya[2], Supriya Sharma, PhD[1*]

[1]Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal
[2]National Public Health Laboratory, Kathmandu, Nepal
[*]Correspondence: supriya.sharma@cdmi.tu.edu.np

Background: Antimicrobial resistance (AMR) is a serious problem to health and development globally. The evidence on AMR among bacteria isolated from hospitals and animals like poultry in Nepal are well documented. However, limited information on the transmission of multidrug resistant bacteria from pigeons and other birds in Nepal are available. Birds live in close proximity to humans, particularly in temples. Kathmandu is also known as the city of temples. Pigeons might carry and transmit pathogenic bacteria through their droppings. Therefore, this study aims to identify aminoglycoside resistant *E. coli* in birds' droppings from selected temples of Kathmandu, Nepal.
Methods: A cross sectional prospective study was conducted for the identification of *E. coli* in birds' droppings at selected temples located within Kathmandu valley. Antibiotic susceptibility testing of the identified bacteria was done by Kirby Bauer Disc diffusion method following CLSI guideline. The *rmtB* gene among the aminoglycoside resistant *E. coli* isolates was detected by conventional PCR.
Results: The predominant bacteria isolated from pigeons' droppings was *E. coli* followed by *Klebsiella* and *Acinetobacter*. About 33% (15/45) of *E. coli* isolates were multidrug resistant. Approximately 31% of *E. coli* were resistant to aminoglycosides and 50% harboured *rmtB* gene.
Conclusions: These results indicate that pigeons could be the reservoir for the transmission of antibiotic resistant bacteria to the environment. Hence, it is urgent to identify alternative approaches to combat antimicrobial resistance.

Keywords: Antimicrobial resistance, Birds' droppings, Temples, Kathmandu, priority pathogens

Oral Presentation 6

Screening of biofilm-producing and antibiotic resistant *Streptococcus mutans* from the oral cavity of smokeless tobacco (Gutkha) chewers in Dharan, Nepal

Kamana Bantawa*, Dhiren Subba Limbu, Srijana Bastola, Bishan Rai

*Department of Microbiology, Central Campus of Technology, Tribhuvan University, Dharan, Nepal *Correspondence: kamana.bantawa@cct.tu.edu.np*

Background: *Streptococcus mutans* is considered as the principal etiological agent of dental caries and has developed multiple mechanisms to colonize the tooth surface. Biofilm also indirectly contributes to bacterial antibiotic resistance by improving the pharmacokinetic properties of the anatomical region. This study looked at whether there would be a connection between the growth of *S. mutans* biofilms and antibiotic resistance among gutkha chewers.

Methods: A total of 235 oral swab samples were collected from the participants aged between 15-64 years, into small container containing sterile phosphate saline buffer and inoculated on Mitis Salivarius Agar. Identification of *S. mutans* was done by colony characteristics, Gram's staining and biochemical tests according to Bergey's Manual. The antibiotic susceptibility test was done by Kirby Bauer disc diffusion and biofilm formation by Microtiter Plate, Tube and Congo Red Agar Method.

Results: The overall prevalence of *S. mutans* was 40%; among them, 92.56% were from male and 7.44% were from female. The highest prevalence of *S. mutans* was found in laborer (52.12%) and the least in government employee (8.51%). ciprofloxacin, bacitracin and kanamycin were resisted by 80.85%, 74.44% and 71.24% of the isolates respectively. In contrast, 96.81%, 71.28% and 69.15% of isolates were sensitive to vancomycin, penicillin and erythromycin respectively. Upon screening of biofilm production, it was observed that 24.65% of the isolates were biofilm producer (6.38% high, 11.70% moderate and 6.38% weak) whereas 75.35% of the isolates were non-biofilm producer. Most of the biofilm producers were resistant to all antibiotics tested. High Biofilm producing isolates were 100% resistant to Ciprofloxacin and Bacitracin but only 33.33% of them were resistant to Vancomycin.

Conclusions: This study demonstrated that *S. mutans* is still the predominant bacterial isolate from the oral cavity. Compared to *S. mutans* biofilm non-producers, *S. mutans* biofilm producers were more resistant to different antibiotics.

Oral Presentation 7

In Silico exploration of flavonoids for dengue therapeutics

Anuraj Phunyal[1], Achyut Adhikari[1*], Jhashanath Adhikari Subin[2*], Rameshwar Adhikari[1,3]

[1]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2]Bioinformatics and Cheminformatics Division, Scientific Research and Training Nepal Pvt. Ltd, Bhaktapur, Nepal
[3]Research Center for Applied Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: subinadhikari2018@gmail.com, achyutraj05@gmail.com*

Background: Dengue virus spreads via the bite of infected *Aedes* species (*Ae. aegypti, Ae. albopictus*) of mosquitoes. Till today there is no approved drug for the cure and prevention of dengue fever. Several inhibitors have been developed by targeting the viral non-structural protein DENV-2 with possible therapeutic values.
Methods: In this work, virtual screening, molecular docking, and molecular dynamics simulations (200 ns) were employed to propose hit candidates from a database of flavonoids (*ca.* 2000 compounds) that may have good preventive potential from the disease.
Results: The binding affinity of flavonoids was determined against protein with a DENV-2. FLD01, FLD02, FLD03, and FLD05 showed binding affinities of −10.2, −10.2, −10.1, −10.1, and −9.9 kcal/mol respectively, and possessed better values than that of the native ligand with showed (−7.6 kcal/mol) and reference drug (−7.3 kcal/mol). Drug likeness of these compounds was acceptable, and no toxicity was hinted by ADMET predictions. The stability of the complexes was accessed from molecular dynamics simulation. Various geometrical parameters, RMSD, RMSF of residues, R_g, SASA, and H-bond count at the end of the production run in the protein-ligand complex support the structural integrity of the adduct. The binding free energy (MM/PBSA) changed up to negative (less than −30.01±7.53 kcal/mol) indicating the spontaneity of the forward reaction and favorability of the product formation.
Conclusions: The results conclude that a flavonoid can bind at the orthosteric site of the target protein of DENV-2 and could inhibit its functioning resulting in the prevention of the disease. A recommendation is made for further experimental and clinical trials of the hit candidate in the quest for effective anti-dengue therapeutics.

Keywords: ADMET, binding free energy, DENV-2, molecular docking, molecular dynamics, RMSD, RMSF

Oral Presentation 8

Screening of phytochemicals as potential anti-breast cancer agents targeting HER2: An *in-silico* approach

Sudarshan Lamichhane[1], Raj Prateek Rai[1], Amar Khatri[1], Rajendra Adhikari[2], Bhupal Govinda Shrestha[1] and Simon Kumar Shrestha[1*]

[1]Department of Biotechnology, Kathmandu University, Dhulikhel, Nepal
[2]Department of Physics, Kathmandu University, Dhulikhel, Nepal
**Correspondence: simon.shrestha@ku.edu.np*

Background: Breast cancer is the most common cancer among women around the world. Human epidermal growth factor Receptor-2 (HER2) is a membrane tyrosine kinase overexpressed in 30% of human breast cancers; thus, it serves as an important drug target. The currently available HER2 inhibitor Lapatinib targets the ATP binding site of the cytoplasmic kinase domain, blocking autophosphorylation and activation of HER-2. However, it causes side effects like diarrhoea, nausea, rash, and possible liver toxicity. As phytochemicals have fewer side effects and are relatively affordable, they offer an effective alternative. Hence, we aimed to identify potential phytochemicals that could act as HER2 inhibitors employing computational methods.

Methods: 1500 phytochemicals from NPACT (Naturally Occurring Plant-based Anti-cancer Compound-Activity-Target) database were docked to the ATP binding site of the HER2 kinase domain using AutoDock Vina, followed by drug likeliness properties prediction based on Lipinski's rule of Five and ADME prediction. The best-docked ligands following Lipinski's rule of five were used for molecular dynamics simulation analysis using GROMACS.

Results: Luxenchalcone, Rhinacanthin Q, Subtrifloralacton D, and 7,7''-dimethyllanaraflavone exhibited higher binding affinity than the reference inhibitor and satisfied the Lipinski's rule of five. Rhinacanthin Q, subtrifloralacton D, and 7,7''-dimethyllanaraflavone formed a stable and compact complex without vast conformational fluctuations with higher mm/pbsa binding energy affinity.

Conclusions: Rhinacanthin Q, subtrifloralacton D, and 7,700- dimethyllanaraflavone could be potential bioactive molecules to act as inhibitors of HER2 protein. Eventually, experimental studies are needed to evaluate the potential of these phytochemicals further. The development of drug discovery can be accelerated using similar computational methods.

Keywords: Breast cancer, HER2, molecular docking, phytochemicals, molecular dynamics simulation

Oral Presentation 9

Insights of 1,3-Dicaffeoylquinate and N-Caffeoyltyramine interactions with SARS-CoV-2 Omicron variant main protease: An *in-silico* study

Kalpana Gyawali, Rajesh Maharjan, Arjun Acharya, Madan Khanal, Madhav Prasad Ghimire, Tika Ram Lamichhane*

Central Department of Physics, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: tika.lamichhane@cdp.tu.edu.np*

Background: The Omicron variant of SARS-CoV-2 spread more rapidly than other variants and affected even vaccinated individuals. The Mpro enzyme was crucial for the replication and transcription of viruses and was also an attractive target for the drug discovery process. This study specifically focused on the *in-silico* investigation for the selection of phytocompounds from medicinal plants against SARS-CoV-2 Omicron variant.

Methods: After studying the ADMET parameters, the selected compounds underwent molecular docking with Mpro, followed by MD simulations to test their stability. The binding mechanisms, including H-bonding, electrostatic, van der Waals and hydrophobic interactions were also analyzed.

Results: The selected compounds demonstrated favorable scores for drug likeness, bioavailability, and pharmacokinetic properties. Compounds 1,3-Dicaffeoylquinate and N-Caffeoyltyramine exhibited binding energies of -6.02 and -6.89 kcal/mol respectively with SARS-CoV-2 Omicron Mpro. Various parameters observed during 50 ns MD simulations showed that the compounds remained stable.

Conclusions: Based on favorable ADMET parameters, binding affinity and stability observed during MD simulations, these compounds could be considered as potential drug candidates against SARS-CoV-2 Omicron variant. Further investigations are needed to prove their effectiveness.

Keywords: SARS-CoV-2 Omicron Mpro, Phytocompounds, ADMET parameters, Molecular docking, MD simulations

Oral Presentation 10

Molecular characterization of extended spectrum beta lactamase producing *Escherichia coli* from clinical specimen

Bindu Ghimire[1*], Zenisha Acharya[1], Milan Uprety[2], Komal Raj Rijal[1], Prakash Ghimire[1]

[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal
[2]Department of Microbiology, Golden Gate College, Kathmandu, Nepal
[*]Correspondence: bindu.ghimire@gmail.com

Background: Antibiotic resistance in pathogenic microorganisms is a paramount stress for the human community. Multidrug resistance (MDR) with extended spectrum beta lactamase (ESBL) progression has brought about a dire situation in the decision-making process of antibiotics use in treatment of *Escherichia coli* (Ec) infection. The purpose of this study was to investigate the prevalence of ESBLEc and its gene types in clinical samples.

Methods: For one year (2020-2021), a cross-sectional study was carried out. All ESBLEc-confirmed isolates were obtained from the National Public Health Laboratory (NPHL), and genotypic analysis was performed at Central Department of Microbiology, Tribhuvan University. Antibiotic susceptibility was determined using Kirby Bauer disc diffusion, and ESBL confirmation was accomplished using the combination disc method. Multiplex PCR was used to confirm the ESBL gene type, and gel electrophoresis was used to visualize the band size.

Results: During the study, 1220 urine samples were analyzed, and substantial bacteriuria was found in 113 of them. Among these *E. coli*, 63 samples were identified, and the prevalence of ESBLEc was assessed to be 28.7%. At the 5% level of significance, there were substantial differences in the antibiotic resistance patterns of ESBLEc and non-ESBLEc. The majority of non-ESBL Ec isolates were resistant to the penicillin category of antibiotics. ESBLEc had 100% resistance to Cefotaxime, Ceftazidime, and Ciprofloxacin drugs, but only 16.7% and 22.2% resistance to Imipenem and Amikacin, respectively. blaCTX-M-1 gene type was recovered from all ESBLEc isolates, 7 of which were identified in conjunction with blaTEM gene type.

Conclusion: The data indicates the presence of *E. coli* and ESBL *E. coli* in the urine sample. The study found that none of the medications used for susceptibility testing were completely effective against *E. coli*. The discovery of the ESBL gene in a DNA extract suggests the possibility of transmission in the environment.

Keywords: Escherichia coli, antibiotic resistance, ESBL

Oral Presentation 11

Hematological and biochemical profiles of patients with dengue virus infection in Lalitpur, Nepal

Indira Rai, MD[1*], Subash Paudel[2], Santosh Shrestha[3], Debbie Manandhar[1],
Shiba Kumar Rai[4]

[1]Asrik Diagnostic Laboratory, Ekantakuna, Lalitpur, Nepal
[2]Trichandra Multiple Campus, Ghantaghar, Kathmandu, Nepal
[3]Nobel College, Sinamanghal, Kathmandu, Nepal
[4]Department of Microbiology and Research Division, Nepal Medical College Teaching Hospital, Gokarneshwor, Nepal
**Correspondence: ena_life5@yahoo.com*

Dengue viral infection is a disease transmitted by mosquitoes of the Aedes genus, and it arises as a result of being bitten by mosquitoes that are carrying the virus. This infection induces a wide range of changes in the body's immune system. Thus, serological evaluation together with hematological and biochemical parameters in cases of dengue viral infection make an accurate clinical diagnosis. The main objective of this study was to study the serological, hematological, and biochemical parameters among patients infected with Dengue virus. A total of 102 blood samples were collected at Asrik Diagnostic Lab, Lalitpur during the period of August to October 2022. Samples were analyzed by following methods: hematological tests were done by coulter counter; Biochemical tests were done by fully automated analyzer and serological tests were done by Dengue test kit. Of the 102 blood samples NS1 antigen was detected in 62 (60.9%) whereas anti- dengue IgM antibody was detected in 10 (9.8%). Leukopenia and thrombocytopenia werepresent in 33 (53.1%) and 32 (51.6%) of the 62 NS1 positive samples, respectively. Of the 62 cases, only 16 samples were tested for the ALT and AST. Out of 16 samples, ALT and AST level were significantly higher in 11 (68.8%) and 14 (87.5%) positive cases, respectively. The statistical analysis showed that there was no significant association between NS1 and IgM. It can be concluded that the investigation of leukocytes, thrombocytes, ALT, and AST helps to diagnose the dengue efficiently.

Oral Presentation 12

Molecular evidence of Leishmania donovani in stray dogs from slum areas of Dharan, Nepal

Dhiren Subba Limbu[1*], Kamana Bantawa[1], Dil Kumar Limbu[1], Prince Subba[1]

[1]Department of Microbiology, Central Campus of Technology,
Tribhuvan University, Dharan, Nepal
**Correspondence: dhirenlimbu2015@gmail.com*

Background: The presence of stray dogs is notable in major urban areas of Nepal, such as Dharan. Most stray dogs endure inadequate access to fundamental healthcare services. The widespread distribution of these stray dogs and a lack of information on canine vector-borne diseases like leishmaniasis in Nepal, this research seeks to assess the occurrence of *Leishmania donovani* in stray dogs residing within various slum areas of Dharan.

Methods: A total of 64 canine blood samples from stray dogs found in 13 different slum areas of Dharan, Nepal from August 2018 to January 2019 were collected on EDTA vials. DNA was extracted by phenol-chloroform method and conventional polymerase chain reaction assays were performed for detection of the 18S rRNA gene of *L. donovani*.

Results: The overall prevalence of *L. donovani* was 7.81 % (5/64). Out of 5 positive cases, 1 (20%) was male and 4 (80%) was female. The presence of *L. donovani* was found higher in female dogs than male dogs but there was no significant relation between the gender of the dog and the prevalence of *L. donovani* (p=0.48).

Conclusions: As far as we are aware, this study marks the initial molecular identification of *L. donovani* in stray dogs inhabiting various slum areas of Dharan, Nepal. The substantial prevalence of *L. donovani* identified underscores the urgency of introducing a surveillance program and implementing control measures for vector-borne diseases among the stray dog population in this region.

Keywords: Dharan, stray dogs, *Leishmania donovani*, 18S rRNA gene, vector-borne disease

POSTER PRESENTATIONS

Poster Presentation 1

Multidrug and ESBL resistant bacteria present in drinking water of Ratnanagar Municipality, Chitwan

Jharana Khanal[1]*, Tika Karki[1], Tista Prasai Joshi[2]

[1]Food and industrial Microbiology, National College, Tribhuwan University, Kathmandu, Nepal
[2]Environment Research Laboratory, Nepal Academy of Science and Technology (NAST), Lalitpur, Nepal
**Correspondence: jharanaa.khanal@gmail.com*

Background: The provision of high-quality drinking water has consistently remained a foremost priority; however, historical microbial contamination, notably coliforms, persists. A substantial portion of the global population still lacks safe water access. Thus, physicochemical as well as microbial safety aspects of drinking water were analyzed. The major motive of this research is to address the serious concern about antibiotic resistance transmission in bacteria through diverse water sources.

Methods: This study was carried out in Ratnanagar Municipality, Chitwan, from February 15 to April 15, 2023. A total of 30 tap water samples were taken and processed following the method described by APHA 2017 and analyzed using the SPSS tool in accordance with the National Drinking Water Quality Standard 2079 and WHO Guidelines.

Results: All the physicochemical parameters were within the standard range. However, 50% iron, and 3.33% turbidity were unsatisfactory level. In microbial analysis, 80% total coliforms, 46.67% faecal coliforms, and 30% total plate count were beyond the water standard. Among 53 bacterial isolates, 13.2% each for Escherichia coli and Shigella, 16.98% Klebsiella, 24.52% Acinetobacter, 18.86% Pseudomonas, 5.67% for Salmonella as well as Citrobacter, 1.28% for Enterobacter and Proteus were confirmed. A total of 12 different types of antibiotics were taken and Antibiotic-susceptible testing was done. Among these 77.35% are positive Extended-Spectrum Beta-Lactamase (ESBL) and 54.71% Multidrug Resistant (MDR).

Conclusion: The result indicates that the drinking water samples analyzed herein could serve as an important source for exposure and dissemination of MDR and ESBL-producing Bacteria, which therewith pose a health risk to the natives as well as tourists residing in Ratnanagar Municipality, Chitwan.

Keywords: ESBL, faecal coliform, MDR, physicochemical parameter, total coliform

Poster Presentation 2

Antimicrobial activity of lichens and effect of silver nanoparticle extracted from lichens against bacterial isolates

Lokraj Awasthi*, Suman Rai*
Department of Microbiology, Amrit campus, Tribhuvan University, Kathmandu, Nepal
**Correspondence: lokrajawasthi4321@gmail.com, suman.rai@ac.tu.edu.np*

Lichens contain bioactive compounds and nanoparticles. Silver nanoparticles in lichens has antibacterial property. Ten lichens species; namely *Ramalina subfarinacea*, *Usnea cornuta*, *Hypotrachyna nepalensis*, *Heterodermia diademata*, *Flavoparmelia caperata*, *Hypotrachyna cirrhata*, *Usnea sp.*, *Usnea orientalis*, *Hypotrachyna cirrhata* and *Ramalina franicea* were identified. Lichen extracts were extracted by Soxhlet method and AgNPs by green synthesis method. AST was done by disc diffusion method, MIC and MBC by microdilution method. Antibacterial property of lichen extracts and AgNPs were tested against *S. aureus* ATCC 25923 and *E. coli* ATCC 35218. All lichen extracts showed antibacterial activity against *S. aureus* ATCC 25923 but none of extracts were active against *E. coli* ATCC 35218. Maximum (13 mm) and minimum (6 mm) ZOI was shown by *H. cirrhata* and *H. diademata* extract respectively, MIC and MBC were 50-200 µl and 200-300 µl against *S. aureus* ATCC 25923 respectively. AgNps was extracted from lichens except *H. diademata* and *F. caperata*. AgNps were active against both bacterial cultures. Maximums (16 mm) and minimum (9 mm) ZOI was shown by *H. cirrhata*. AgNps. MIC and MBC were 75-200 µl and 100 –300 µl against *S. aureus* ATCC 25923 respectively. Against *E. coli* ATCC 35218; maximum (12 mm) and minimum (8 mm) ZOI by *U. cornuta* and *H. cirrhata* AgNps respectively. MIC and MBC were 100-200 µl and 200-300 µl respectively. Lichen extracts and AgNps have effective antibacterial activity against *S. aureus* ATCC 25923 than *E. coli* ATCC 35218.

Keywords: AgNps, antibacterial activity, Lichen, MBC, MIC.

Poster Presentation 3

Beta-lactamase producing genes in Gram-negative bacteria isolated from clinical specimens

Ashish Parajuli[1*], Aruna Khanal[1], Anil Kumar Sah[2], Jeena Amatya[3]

[1]St. Xavier's College, Tribhuvan University, Kathmandu, Nepal
[2]Annapurna Research Center, Kathmandu, Nepal
[3]Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu Nepal
**Correspondence: ashparap@gmail.com*

Background: Extended-spectrum beta-lactamase (ESBL)-producing organisms show resistance towards most beta-lactam antibiotics, including penicillins, cepaholsporins, and monobactam. Similarly, metallo-beta-lactamases (MBLs) confer resistance against carbapenems. This study was carried out to detect the presence of ESBL-producing gene ($\textit{bla}_{\text{CTX-M}}$) and MBL-producing gene ($\textit{bla}_{\text{VIM}}$) in Gram-negative bacteria isolated from clinical samples.

Methods: A total of 459 samples were taken for this study. The study period was from February to August 2021, which was carried out in Annapurna Neurological Institute and Allied Sciences, Kathmandu, Nepal. Screening and phenotypic confirmation of ESBL was done by modified Kirby-Bauer disk diffusion method, following the CLSI (2018) guidelines. Screening and phenotypic confirmation of Gram-negative isolates for the presence of the beta-lactamase genes was done. Phenotypically confirmed ESBL and MBL producing bacteria were considered for gene detection. Alkaline lysis method was used to extract plasmid DNA from Gram-negative isolates.

Results: Amikacin and imipenem were found to be the most effective drug against Gram-negative isolates. Of 138 isolates, 95 (68.8%) isolates were screened as ESBL-producers, whereas 38 (27.5%) isolates were screened as MBL-producers. Of the 95 isolates, 48 (51%) were phenotypically confirmed, from which 41 (85.4%) isolates were found to be carrying $\textit{bla}_{\text{CTX-M}}$ gene. Of the 38 isolates, 28 (73.7%) isolates were phenotypically confirmed as MBL producers, among which 5 (17.9%) isolates were found to be carrying $\textit{bla}_{\text{VIM}}$ gene. Both, $\textit{bla}_{\text{CTX-M}}$ gene (29.3%) and $\textit{bla}_{\text{VIM}}$ (80%) gene were found highest in *Klebsiella pneumoniae*, in this study.

Conclusion: The data obtained from this study suggests that the increasing cases of $\textit{bla}_{\text{CTX-M}}$ and $\textit{bla}_{\text{VIM}}$ gene pose a serious threat in treatment of bacterial infections.

Keywords: Gram-negative bacteria, AST, ESBL, MBL, $\textit{bla}_{\text{CTX-M}}$, $\textit{bla}_{\text{VIM}}$

Poster Presentation 4

Green Synthesis, characterization of silver nanoparticles using *Nymphaea nouchali* Burm. f and its antioxidant and antimicrobial activities

Asmita Rayamajhi, Nisha Gyawali, Achyut Adhikari*

Central Department of Chemistry, Tribhuvan University, Kirtipur, Nepal.
**Correspondence: achyutraj05@gmail.com*

Background: The green route biosynthesis of nanoparticles is an innovative strategy for nanotechnology that offers an economical and environmentally friendly substitute for physical and chemical approaches. *Nymphaea nouchali* is an essential herb that has been used traditionally for a variety of purposes, from biological to nanomaterial. The aim of this study is to investigate an efficient and sustainable route of AgNPs using rhizome extracts and to evaluate its antioxidant and antimicrobial activity.

Methods: AgNPs were synthesized by combining plant extract in a 1:4 ratio with 1 mM AgNO3 at room temperature. The color change, UV-vis spectrometer, XRD, FTIR, and SEM image are used to characterize the biosynthesized AgNPs. Additionally, the antibacterial activity was assessed using the agar-well diffusion method and the DPPH assay for antioxidants.

Results: A UV-visible spectrophotometer and scanning electron microscopy were employed to characterize the AgNPs. Additionally, Fourier transform infrared spectrometer (FTIR) analysis was conducted to identify the composition of the capping agents present in the extract. The results from antioxidant assays confirmed that the AgNPs exhibited a higher inhibition percentage in comparison to the plant extract. Furthermore, the obtained AgNPs demonstrated significantly elevated antimicrobial activities *against E. coli* (with a zone of inhibition (ZOI) of 3.8 mm and minimum inhibitory concentration (MIC) of 172 µg/mL) and *B. subtilis* (ZOI=4 mm and MIC=120 µg/mL) when compared to the plant extracts.

Conclusions: This work enhances antibacterial activity and opens up further research in this field. This plant extracts possess potent compounds with antioxidant and antibacterial properties that can be used as an agent in designing and developing new drugs.

Poster Presentation 5

Identification of natural phytoecdysteroids as ROS1 inihibitor using *in-silico* approach

Bishnu Adhikari[1], Rabindra Adhikari[1], RameshwarAdhikari[1,3], Achyut Adhikari[1*], Jhashanath Adhikari Subin[2*]

[1]Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal
*[2]Bioinformatics and Cheminformatics Division, Scientific Research and Training Nepal P. Ltd, Bhaktapur, Nepal [3]Research Center for Applied Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal *Correspondence: achyutraj05@gmail.com*

Background: Lung cancer is a type of cancer that causes the most deaths worldwide. Till date there is no specific drug for the cure and prevention of lung cancer. ROS1 is tyrosine kinase receptor that causes different types of cancers including lung cancer. A kinase protein was selected with PDBID of 3ZBF as a good target for therapeutic measures.

Methods: In this work, protein preparation, ADMET analysis, molecular docking, and server based molecular dynamics simulations were employed to suggest potential hit candidate from the collection of phytoecdysteroids (*400* compounds) that may have good preventive potential for lung cancer.

Results: The binding affinity of Phytoecdysteroids was determined against protein with PDB ID 3ZBF.PES40, PESPES33, and PES29 are the top three candidates which showed better binding affinities of −9.2, −8.8 and −8.5 kcal/mol respectively, and possessed better values than that of the native ligand with showed (−8.4 kcal/mol) and reference drug (−7.0 kcal/mol). Drug likeness of these compounds was acceptable, and no toxicity was found except immunotoxicity by ADMET predictions. The stability of the adduct was accessed from server based molecular dynamics simulation.

Conclusions: The results conclude that a phytoecdysteroids can bind at the active site of the target protein of ROS1 and could inhibit its functioning resulting in the prevention of the cancer. A recommendation is made for further experimental and clinical trials of the hit candidate for effective lung cancer therapeutics.

Keywords: ADMET, ROS1, molecular docking, molecular dynamics, RMSF

Poster Presentation 6

Extraction of nanoparticles by green synthesis of *Physica aipolia* (Ehrh.ex Humb.) Furnr and its application in biological activity

Deepa Karki, Asmita Sapkota, Achyut Adhikari*

Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal
**Correspondence: achyutraj05@gmail.com*

Background: In the present study, an eco-friendly method of green synthesis of AgNPs was carried out using the aqueous extract of *Physica aipolia* which acts as both capping and reducing agents. Lichens have evolved into a rich source of innovative bioactive chemicals and their antioxidant capabilities, widening the scope of well-documented and effective anti-microbial activity.
Methods: AgNPs were prepared by adding lichen extract in a 1:4 ratio with 1mM $AgNO_3$ at room temperature. Different instruments were used to characterize the green synthesized nanoparticles (UV-vis spectrometer, XRD, FTIR, and SEM). Furthermore, the Agar-well diffusion method was also carried out to demonstrate anti-bacterial activity free radical scavenging activity was performed by DPPH assay.
Results: The UV-Vis spectroscopy showed a characteristic SPR band of Ag at 418 nm indicated the formation of AgNPs and were found to be stable for more than a month. The AgNPs were face centered cubic (fcc) crystalline with average size of 7 nm as calculated from the XRD data. The SEM images revealed the spherical and polycrystalline AgNPs within the agglomerated form. The FTIR spectra elucidated the broad strong peak at 3632 cm^{-1} extract was weak in nanoparticles indicating the involvement of -OH group of compounds of extract in the reduction of silver ions. For antioxidant inhibition activity crude extract of methanol showed IC_{50} value of 5.324 ± 0.80 µg/mL. While performing anti-bacterial, crude extract against *Staphylococcus aureus 29213* with 11 mm mg/mL.
Conclusions: The outcome of these findings suggests the application of *Physica aipolia* influenced AgNPs for biological activity.

*Keywords***:** Nanoparticle, Silver nanoparticle, Anti-bacterial, green synthesis, Antioxidant.

Poster Presentation 7

Anti-cancer activity of zinc oxide nanoparticles in photodynamic therapy

Crishpa Budha Magar [1], Jharna Bajracharya [1], Abhaya Rajkarnikar [1], Aarati Upreti [1], Palpa Paudel [1], Rishi Baniya [1], Rameshwar Adhikari [2,3] *

[1]College of Biomedical Engineering and Applied Sciences, Purbanchal University, Kupondole, Lalitpur, Nepal
[2]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal
*[3]Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kirtipur, Kathmandu, Nepal *Correspondence: nepalpolymer@yahoo.com*

Background*:* Extensive research in the field of nanotechnology has significantly facilitated the assessment of cancer severity through a highly specific alternative treatment approach known as photodynamic therapy (PDT). It uses a combination of nanoparticles, photosensitizers, and light of a specific wavelength. Among various nanoparticles, Zinc Oxide nanoparticles (ZnO-NPs) act as a photosensitizer and trigger a photodynamic reaction upon activation by UV light. It destroys cancer cells through the production of reactive oxygen species (ROS).

Methods: ZnO-NPs were synthesized through two distinct methods: the *sol-gel* method and a biologically assisted approach utilizing pumpkin seed extract. The ZnO-NPs underwent thorough characterization using various analytical methods, including ultraviolet-visible spectroscopy (UV-Vis spectroscopy), Zeta potential, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). The light source for PDT was built using 96 UV LEDs in a printed circuit board (PCB).

Results: ZnO-NPs were rod-shaped with a hexagonal wurtzite crystal structure, an absorption peak at 376 nm, and a size range of 30nm - 85 nm. Zeta potential values for chemically and biologically assisted synthesized ZnO-NPs were determined as -145 mV and -120 mV, respectively. The result of the MTT assay revealed a dose-dependent reduction in cell viability upon treatment with various concentrations of ZnO-NPs and exposure to UV light. Additionally, a time-dependent suppression of cell viability was observed with UV irradiation. Notably, no statistically significant difference was observed in the viability of MCF-7 cells when treated with ZnO-NPs synthesized through both methods.

Conclusion: Our overall findings suggest that ZnO-NPs synthesized through different methods exhibit promising anticancer potential against the MCF-7 cell line when employed in photodynamic therapy (PDT). Emphasizing the diverse applications and versatility of ZnO-NPs.

*Keywords***:** photodynamic therapy, photosensitizer, zinc oxide nanoparticle, anti-cancer

Poster Presentation 8

Preliminary structural characterization of commercial *Ayurvedic Bhasmas* as nanoformulation

Bimal Rajchal[1, 2, 3], Pramod Bhatta[4], Rameshwar Adhikari[1, 3]*

[1] Central Dept. of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2] Dept. of Chemistry, Bhaktapur M. Campus, Tribhuvan University, Bhaktapur, Nepal
*[3] Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kirtipur, Kathmandu, Nepal [4]Ayurveda Campus and Teaching Hospital, Institute of Medicine (IoM), Kirtipur, Kathmandu, Nepal *Correspondence: nepalpolymer@yahoo.com*

Background: *Ayurvedic Bhasmas* are herbo-mineral/metallic nano-formulations that are employed as effective medications in Ayurvedic treatment. The preparation process for these formulations is very similar to the top-down approach to nanoparticle synthesis. These are usually made by repeatedly triturating minerals or metals with extracts of potent ayurvedic herbs and then calcining at temperatures above 650 °C. These compositions are regarded as nano-drugs, and compared to equivalent mineral or metal nanoparticles, they are more enriched with a variety of metabolites and have improved medicinal properties. The exceptional structural dimensions and properties of these particles may have potential nanomedicine applications in the sectors like management of chronic illness, drug delivery, wound healing, regenerative medicine, and therapy development.

Methods: Different kinds of *Ayurvedic Bhasma* samples (such as *Heerak Bhasma, Rajat Bhasma, Abhrak Bhasma, Yashad Bhasma, Vanga Bhasma, Swarnamakshik Bhasma*) were collected from the market and subjected to UV-visible and Fourier transform infrared (FTIR) spectroscopic studies under ambient conditions.

Results: The UV-visible spectroscopy of commercial *Ayurvedic Bhasmas* showed absorption peaks in the range of 304 nm to 365 nm indicating their nanoscale dimensions. The FTIR results of these *Bhasma* samples displayed the presence of varieties of stretching (O-H, C-H, C=O, N-O, S=O) and bending (C-H, C=C) organic moieties.

Conclusion: The commercial *Bhasma* samples contain very fine particles of nanometer size but these particles displayed agglomeration and polydispersity. The samples were also found to be enriched with various organic groups that enhance their medicinal properties.

Keywords: Ayurvedic herbs, medications, nano-formulations, nanomedicine

Poster Presentation 9

Investigation of antibacterial properties of essential oil extracted from *Hyptis suaveolens (Ban-Tulsi)*

Ikcha Shahi[1], Elisha Dongol[2], Nelson Rai[1], Manish Man Shrestha[1], Sabina Shrestha[3], Rameshwar Adhikari[1, 2]*
[1]Central Department of Chemistry, Tribhuvan University, Kirtipur 44618, Kathmandu, Nepal
*[2] Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kirtipur 44618, Kathmandu, Nepal [3]Madan Bhandari University of Science and Technology, Chitlang, Thaha Municipality – 9, Makwanpur, Nepal *Correspondence: nepalpolymer@yahoo.com*

Background: *Hyptis suaveolens* is one of the highly invasive plant species found in Nepal, but native to tropical America. It has a mild aroma that resembles the *Ocimum sp.* (Tulsi) plant, therefore, the local name Ban-Tulsi. In the present work, the antibacterial properties of essential oil extracted from aerial parts of *Hyptis suaveolens* were investigated against one Gram-positive bacterium, *Staphylococcus aureus,* and three Gram-negative bacteria, *Escherichia coli*, *Pseudomonas sp.*, and *Klebsiella pneumoniae.*

Methods: Plant samples were collected from Baraha-03, Sunsari district, and shade-dried. Dried samples were ground into fine powder. The powder was used to extract essential oil by hydro-distillation technique using Clevenger apparatus. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used for phytochemical screening of essential oil. The disc diffusion method was followed to study essential oil's antibacterial activities. The discs, 6 mm in diameter each, were prepared on Whatman No. 1 filter paper for the absorption of essential oil to be tested for its antibacterial properties. The essential oil sample for the antibacterial test was prepared by mixing 40 μL oil and 60 μL dimethyl sulfoxide (DMSO). A serial microdilution assay as a microbial growth indicator was used to evaluate the minimum inhibitory concentrations (MICs). The samples of different concentrations were soaked in the disc and placed on the surface of the microbial-seeded agar plate. Then the plates were incubated at 37 °C for 24 hours and the zones of inhibition were measured.

Results: Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed the highest percentage of Caryophyllene (30.4 %). The antibacterial activities were recorded by measuring the zone of inhibitions. MICs for *E. coli*, *Pseudomonas sp.*, and *K. pneumoniae* 4 μg/mL, 40 μg/mL and 4×10^4 μg/mL respectively. However, there was no inhibition zone observed for *S. aureus*. This may be due to the thick peptidoglycan layer of gram-positive bacteria and the low concentration of essential oil.

Conclusions: The results showed good antibacterial properties against Gram-negative bacteria. In addition to this, the essential oil may be effective against Gram-positive bacteria by increasing the concentration of the oil.

*Keywords***:** Hydro-distillation, Microdilution, MIC, Clevenger apparatus, GC-MS

Poster Presentation 10

Effectiveness of antihelminth against ascariasis infection in slum area of Butwal, Nepal

Shristi Bhandari, Kishor Pandey*
Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: kishor.pandey@cdz.tu.edu.np*

Background: Intestinal parasitic infection is the most widespread of human infections, primarily in developing nations like Nepal. Increasing urbanization results in the formation of slum communities along with health issues primarily helminth infection due to inferior quality of living. This study aims to implement proper medication in people positive for ascariasis.

Methods: A total of 70 faecal samples were collected from the slum area of Butwal by convenient sampling method. Direct smear and concentration method was used to examine the samples for *Ascaris lumbricoides.* Albendazole was given to the people with positive samples (N=25) and the fecal samples were recollected after one week of administration and re-examined for possible parasitic infection.

Results: The prevalence of *A. lumbricoides* in the first case was 35.71%. Following medication, the drug's efficacy was found to be 84% and the prevalence was found to be 16%.

Conclusions: The study concluded that a single dose of albendazole plays a vital role in being the first choice of anthelminthic medication for Ascariasis with low infections. Due to high intensity of *A. lumbricoids* infection, the prevalence continued even after treatment. This indicates that a regular albendazole dose is necessary to treat high parasite infections. *Keywords***:** Slums, parasites, prevalence, albendazole, ascariasis

Poster Presentation 11

Analyzing tuberculosis type distribution and prevalence by gender and age groups: A year-long study at Dhaulagiri Zonal Hospital, Baglung, Nepal

Anisha K.C., Kishor Pandey*, Laxman Khanal
Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: kishor.pandey@cdz.tu.edu.np*

Background: This study addresses the influence of gender and age group on the prevalence or distribution of disease type. It aims to determine the TB dynamics, distribution of TB types and the prevalence across different genders and age groups at Dhaulagiri Zonal Hospital, Baglung, Nepal.

Methods: Data was extracted from the hospital's records from September 2022 to August 2023 A.D. This data included details on presumptive TB cases, encompassing patient demographics like age, gender and disease type. Data analysis was done in MS-Excel and R-software.

Results: This study examined 812 suspected TB cases, comprising 412 males and 400 females, with 28 confirmed cases. TB was in higher prevalence among males than females ($p>0.005$), with the highest prevalence in 15-64 years age group and the lowest in 0-14 years group. The majority had Pulmonary Bacteriologically Confirmed (PBC) TB (n=17), followed by Extra Pulmonary (EP) TB (n=9) and Pulmonary Clinically Diagnosed (PCD) TB (n=2). Chi-square tests indicated no significant association between TB disease type and gender ($p>0.005$) or age ($p>0.005$).

Conclusions: The absence of significant association between gender and prevalence indicates that factors beyond gender, such as environment, genetics, lifestyle, or occupational exposure likely contribute significantly to the observed differences in TB prevalence between males and females. The predominance of PBC TB without significant gender or age highlights the need for targeted interventions in this region. *Keywords***:** Tuberculosis, Nepal, Prevalence, age, gender

Poster Presentation 12

Effectiveness of helminthic parasite control in *Ovis aries* of Chandragiri

Sandhya Gautam, Kishor Pandey*
Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: kishor.pandey@cdz.tu.edu.np*

Background: The gastrointestinal health of *Ovis aries* (sheep) is critical to both animal welfare and agricultural productivity small ruminants, such as sheep, commonly face challenges from helminthic parasites, contributing to issues ranging from disease susceptibility to a decline in meat and wool production. This research aims to assess the effectiveness of administering anti-helminthic drugs in controlling helminthic parasites in *Ovis aries*.

Methods: Faecal samples of sheep were collected from the Magar village of Chandragiri through a convenient sampling method, accompanied by a questionnaire. The samples underwent examination using both direct smear and concentration methods. Sheep with positive samples received fenbendazole, and faecal samples were collected again one-week post-administration for re-examination to detect any potential parasitic infections.

Results: Four different species were observed *Haemonchus (*35.29%)*, Trichostrongyloides* (17.64%), *Oesophagostomum* (23.52%)*,* and *Strongyloides* (23.52%). However, persistence of species *Strongyloides* was seen after treatment. The Helminthic parasite prevalence stood at 85% prior to treatment; post-medication, it dropped to 23.5%. The medication exhibited effectiveness in 76.47% of the samples.

Conclusions: The study concluded that a single dose of fenbendazole may not be adequate for anthelminthic treatment; instead, administering multiple doses to sheep is recommended for a more effective reduction of helminthic parasites. *Keywords***:** *Ovis aries*, Helminthic parasite, fenbendazole.

Poster Presentation 13

Prevalence of trematode parasites in the liver of buffaloes brought to slaughterhouses in Bhaktapur

Anju Karmacharya, Janak Raj Subedi*
Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: janzoology@gmail.com*

Background: The liver is one of the vital organs of the body which is susceptible to various parasites that affect the total health status of buffaloes. This study was carried out to determine the prevalence of liver parasites in slaughtered buffaloes in Bhaktapur abattoirs.

Methods: Altogether 70 livers were examined from Sep 30 to Oct 25, 2023, by macroscopic and postmortem examination. The infected liver was preserved in 70% alcohol and the adult flukes were extracted from the liver. The flukes were kept in soda water to relax their body, following which morphometric measurements of the parasites such as body length (BL), Body width (BW), Acetabulum diameter, BL/BW ratio, the distance between ventral sucker and posterior end of the body were taken.

Results: A total of 21.24% prevalence was detected with 14.28% *Gigantocotyle explanatum*, 5.71% (*Fasciola gigantica* + *Gigantocotyle explanatum*) and 1.43% *Fasciola gigantica*. The buffaloes brought from Jitpur showed a higher prevalence i.e. 40%. The average length of flukes is 3.56cm (*F. gigantica*) and 0.84cm (*G. explanatum*).

Conclusion: This study emphasizes the need for effective parasite control in the liver of buffalo for better meat quality in slaughterhouses. Meat inspection in slaughterhouses is highly recommended for better meat quality. *Keywords***:** Buffalo, Bhaktapur, Liver, Parasite

Poster Presentation 14

Epidemiological impact of temporal climatic variability on dengue in Kathmandu, Nepal

Niten Bharati, Kishor Pandey*
Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: kishor.pandey@cdz.tu.edu.np*

Background: This study addresses the poorly explored relationship between climatic factors and dengue in Nepal. It aims to identify environmental factors influencing dengue incidence in Kathmandu, developing a forecasting model.

Methods: Monthly dengue fever cases (January 2022 to October 2023) were obtained from the Epidemiology and Disease Control Division (EDCD), and weather data from Department of Hydrology and Meteorology (DHM) Kathmandu Airport stations. Lagged cross-correlation and negative binomial regression, addressing overdispersion, were applied for modeling and future case prediction. Statistical analysis used R version 4.3.2.

Results: There is a positive correlation between climatic variables and dengue cases, with the effect becoming evident at a lag of 1 to 3 months. Precipitation ($p<0.001$), relative humidity($p<0.001$) and minimum temperature ($p<0.001$) emerge as the best predictors. A one-unit rise in minimum temperature increases the relative risk by 1.6 units. Integrating climate variables into negative binomial regression enhances predictive power for forecasting 2023 cases.

Conclusions: This model has the potential to be utilized for monitoring and predicting dengue incidence in the Kathmandu district, offering valuable insights for public health management.

Keywords: Dengue, climate, cross-correlation, Negative binomial regression, forecasting

Poster Presentation 15

Green synthesis of silver nanoparticles using *Curcuma Longa* extract and its anti-microbial activity

Asmita Sapkota[1], Deepa Karki[1], Supriya Sharma[2], Achyut Adhikari[1*]
*[1]Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal [2]Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal *Correspondence: achyutraj05@gmail.com*

Background: The green synthesis approach was employed in this study for the preparation of silver NP's using the aqueous extract of *Curcuma longa* rhizome. The metabolites present in *C. longa* pay important part in the reduction of $AgNO_3$ to Ag and capping of silver nanoparticles.

Methods: AgNPs were prepared by the green synthesized using *C. longa* as an extract in a 1:4 ratio with 1mM $AgNO_3$ at room temperature. The characteristics of silver nanoparticle was analyzed by using UV-visible spectrometer, FTIR, XRD spectra which provided insights into the capability for the characterized Ag nanoparticle. Moreover, the Agar-well diffusion method was used to demonstrate the anti-microbial activity.

Results: The confirmation of AgNPs formation was observed through a characteristics SPR band of Ag at 428 nm in UV-Visible spectroscopy and these were found to be stable for more than 20 days. The AgNPs were face-centered cubic (fcc) crystalline, predicted from the XRD data having an average size of 6.66 nm. The strong and wide peak at 3632 cm^{-1} observed in the rhizome extract was significantly weakened in the nanoparticles, suggesting the participation of hydroxyl group (-OH) present in the extract's compounds in the reduction of silver ions in FTIR. Furthermore, agar well diffusion showed the zone of inhibition of 8 mm against *E. coli* and 10 mm *against S. aureus*.

Conclusions: These results suggest an application of AgNPs influenced by *Curcuma longa* for anti-microbial inhibition activity.

*Keywords***:** Green synthesis, Nanoparticle, Silver nanoparticle, Anti-microbial, ZOI

Poster Presentation 16

Antibacterial properties of essential oil extracted from *Polygonum hydropiper* (Pirre Jhaar*)*

Nelson Rai[1], Manish Man Shrestha[1], Ram Prasad Baral[1], Elisha Dongol[2], Rajesh Pandit[3]*
[1]*Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal*
[2]*Research Centre for Applied Science and Technology (RECAST), Kathmandu, Nepal*
[3] *Department of Chemistry, Tri-Chandra Multiple Campus, Kathmandu, Nepal*
**Correspondence: panditrajesh02@gmail.com*

Background: Herbal plants are gaining tremendous attention of scientific community because of its medicinal values. *Polygonum hydropiper* locally known as "Pirre jhaar" is an aromatic plant used as ethno-medicine. The present study aimed to investigate antibacterial properties of essential oil obtained from aerial parts of the plant against one gram-positive bacteria- *Staphylococcus aureus* and three gram-negative bacteria- *Escherichia coli*, *Pseudomonas sp*., and *Klebsiella pneumoniae*.

Methods: Plant samples were collected from different places around Kathmandu valley and shade dried. Dried samples were grinded into fine powder. The powder was used to extract essential oil by hydro-distillation technique using Clevenger apparatus. Gas Chromatography Mass Spectrometry (GC-MS) technique was employed for phytochemical screening and analysis. A serial microdilution assay as a microbial growth indicator was used to evaluate the minimum inhibitory concentrations (MICs). Samples of different concentrations were soaked in disc and placed on the surface of the microbial-seeded agar plate. All the plates were then incubated at 37 °C for 24 hours. The antibacterial activity of the essential oil was recorded by measuring the zone of inhibition.

Results: GC-MS analysis showed highest percentage of Piperitone (32.09 %) and Terpinene (16.59 %) in the essential oil. MICs of the essential oil for *S. aureus*, *E. coli*, *Pseudomonas* sp., and *K. pneumoniae* were 7×10^{-1} μg/mL, 7×10^{-2} μg/mL, 7×10^{-1} μg/mL and 7×10^{4} μg/mL, respectively.

Conclusions: The essential oil of *P. hydropiper* showed antibacterial activities against both gram positive and gram-negative bacteria.

*Keywords***:** Hydro-distillation, Microdilution, MIC, GC-MS

Poster Presentation 17

Holy Basil (*Ocimum sanctum*): Exploring the chemistry and antibacterial activity of the essential oil from a traditional medicinal plant via modern technology

Manish Man Shrestha[1], Nelson Rai[1], Ram Prasad Baral[1], Elisha Dongol[2], Rajesh Pandit[3]*
[1]*Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal*
[2]*Research Center for Applied Science and Technology (RECAST), Kathmandu, Nepal*
[3]*Department of Chemistry, Tri-Chandra Multiple Campus, Kathmandu, Nepal*
**Correspondence: panditrajesh02@gmail.com*

Background: Novel biomolecules utilized extensively in ethnomedicine can be found naturally in herbal plants. *Ocimum sanctum,* commonly known as Tulasi or Tulsi in Nepali and Holy Basil in English, is an aromatic plant in the family Liliaceae. Tulsi/Basil leaves have been traditionally used for the treatment of many infections. Basil leaves and branches are frequently used as insect repellents and insecticides against mosquitoes, bees, flies, and other insects. It is reported to have numerous bio-physiological activities, including activity against acute peptic ulcer as well as gastric cytoprotective antiulcer, antifungal, and antibacterial activity. The present study aimed to investigate the antibacterial properties of essential oil of *O. sanctum* against a gram-positive bacterium (*Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Pseudomonas* sp., and *Klebsiella pneumoniae*).

Methods: Tulsi leaves were collected from different places around Kathmandu district and shade-dried. Dried samples were ground into fine powder. Essential oil was extracted by hydro-distillation technique using Clevenger apparatus. Gas Chromatography-Mass Spectrometry (GC-MS) was employed for the phytochemical analysis. Disc diffusion method was used to determine the antibacterial activity of the essential oil. Serial microdilution assay as a microbial growth indicator was used to evaluate the minimum inhibitory concentrations (MICs). Samples of different concentrations were soaked in the disc and placed on the surface of the microbial-seeded agar plate. The plates were incubated at 37 °C for 24 hours and the antibacterial activity of the essential oil was recorded by measuring the zone of inhibition.

Results: GC-MS analysis revealed the maximum presence of Anethole and Linalool in the essential oil, 49.00% and 28.17%, respectively. MIC of the essential oil for *S. aureus*, *E. coli*, *Pseudomonas* sp., *and K. pneumoniae* was 7 µg/mL, 7×10^{-2} µg/mL, 7×10^{2} µg/mL, 7×10^{2} µg/mL, respectively.

Conclusion: The essential oil of *O. sanctum* has antibacterial activity against both gram-positive and gram-negative bacteria.

Keywords: Hydro-distillation, microdilution, MIC**,** GC-MS, Anethole, Linalool

Poster Presentation 18

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing among Methicillin- Resistant *Staphylococcus aureus* (MRSA) from hospital environment and postoperative patients

Krishma Pandit[1], Prativa Poudel[1], Sanjib Adhikari[1], Srijana Koirala[2], Supriya Sharma[1,]*
[1]*Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal*
[2]*Bhaktapur Hospital, Bhaktapur, Nepal *Correspondence: supriya.sharma@cdmi.tu.edu.np*

Background: Methicillin resistant *S. aureus* (MRSA) has emerged as one of the most important nosocomial pathogens. Resistance to methicillin is due to acquiring the *mecA* gene which is usually carried on a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). This study aims to determine the prevalence of MRSA, detection of methicillin resistant gene *mecA* and characterize SCC*mec* types in MRSA from post-operative patients and hospital environment.

Methods: This hospital based cross sectional study was carried out among clinical samples collected from post-operative patients and environmental samples including swabs from inanimate surfaces and air samples from different wards of hospital. Each sample was processed for isolation of MRSA by culture and *mecA* gene. SCC*mec* typing was done conventional PCR.

Results: From clinical samples, out of 29 *S. aureus* isolates, 20 (68.9%) were MRSA. From environment samples, out of 33 *S. aureus* isolates, 22 (66.7%) were MRSA. *mecA* was detected in more than 85% of clinical isolates and about 72% of environmental isolates. SCC*mec* type II followed by type V and I was predominant among the isolates from both environmental and clinical samples. However, SCC*mec* Type III, Type Iva, Type IVb, Type IVc and Type IVk were not detected in both clinical and environmental samples.

Conclusion: SCC*mec* elements were present in both post-operative patients and hospital environment at the same time. Therefore, proper preventive methods should be implemented to break the chain of transmission of MRSA from hospital environment to patients and vice versa.

Keywords: MRSA*; SCCmec; Staphylococcus aureus;* post-operative ward

Poster Presentation 19

Prevalence of *Salmonella* infection among the suspected enteric fever patients conducted at a secondary hospital in Kathmandu, Nepal

Santosh Kumar Gupta[1*], Khushbu Shah [2]
[1]*School of Health and Allied Sciences, Pokhara University, Pokhara-30, Kaski, Nepal*
[2]*Manipal College of Medical Sciences, Pokhara, Nepal*
Correspondence: skgupta7124@gmail.com

Background: Enteric fever is one of the common public health problems in Nepal. The progressive increase in antibiotic resistance among pathogens in developing countries is becoming a critical area of concern globally. The present study was carried out at Everest Hospital, Kathmandu, Nepal, with an objective to determine the prevalence of enteric fever and to analyze the status of antimicrobial resistance pattern of *Salmonella enterica* serovar Typhi and Paratyphi isolated from blood specimens. Blood samples collected were used for Total Leucocyte Count and cultured in Brain Heart Infusion broth. If followed with turbidity, subcultured and antibiotic susceptibility testing was performed. Identification of the isolates was done by standard microbiological techniques and antibiotic susceptibility testing was done by modified Kirby Bauer disc diffusion method following clinical and laboratory standard guidelines (CLSI).

Methods: A prospective study was carried out at Everest Hospital, Kathmandu, Nepal from April 2016 to December 2014 on patients having a febrile episode lasting for ≥ 3 days presenting them at Everest Hospital requesting for blood culture and susceptibility testing. Cases included in the study were patients defined by physicians as probable cases of enteric fever with fever (38°C and above) that has lasted for at least three days and showing clinical signs and symptoms of enteric fever. A total of 692 blood samples from patients following case definition of suspected enteric fever were included in the study. Samples with improper labeling, insufficient blood volume, and inappropriate collection and transport were rejected.

Results: Among 692 samples, 40(5.8%) samples were found to be culture positive whereas 652 (94.2%) cases were culture negative. Out of 40 isolates, 22 (55%) were *S*. Typhi and 18 (45%) were *S*. Paratyphi A and the prevalence was higher in male and in the age group of 11-20 years. All the *S*. Typhi infected cases showed leukocytosis while *S*. Paratyphi infected cases showed the total leucocyte count (TLC) within normal range. Most effective antibiotics were chloramphenicol and cotrimoxazole. Among the 22 *S.* Typhi isolates, 21 (95.5%) were nalidixic acid resistant (NAR). Out of 18 *S.* Paratyphi A isolates, 16 (88.9%) were nalidixic acid resistant. Among 21 nalidixic acid resistant (NAR) *S.* Typhi, only 2 isolates were found to be resistant to ofloxacin and only 11 isolates were found to be sensitive to ciprofloxacin. Similarly, out of 16 isolates of *S.* Paratyphi A resistant to nalidixic acid, only one of the isolates was found to be resistant to both ofloxacin and ciprofloxacin.

Conclusion: It was concluded that higher rate of nalidixic acid resistance as well as emerging resistant pattern to other fluroquinolone antibiotics suggests us to follow correct treatment regimens and good infection control practices.

Keywords. Enteric fever, *S*almonella species, NAR, BHI, TL

Poster Presentation 20

Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical specimens

Chandra Kant Yadav
School of Health and Allied Science, Pokhara University, Pokhara, Nepal.

Background: *Staphylococcus aureus (S. aureus)* is a commensal as well as pathogenic bacteria that causes leading human Skin and soft tissue infection worldwide. Increasing resistance to antimicrobial agents among *S. aureus* causes problems in the treatment. This led to the usage of macrolide Lincosamide and Streptogramins B (MLSB) group of antibiotics for the treatment of *S. aureus* infection. Routine antibiotic susceptibility Test (AST) in vitro test fails to detect inducible clindamycin resistance (ICR) due to *erm* gene as a result treatment failure. The aim of this study was to determine the prevalence of inducible clindamycin resistant *S. aureus* isolated from clinical specimens**.**

Methods: Seventy (70) *S. aureus* was isolated from the various clinical samples at the Department of Microbiology, Galaxy Hospital, Pokhara. AST was performed to all *S. aureus* isolates, including Cefoxitin (30mcg) by Kirby-Bauer disk diffusion method. ICR in *S. aurues* was detected by D-Test. The data analysis was performed in MS-Excel and SPSS software V25.0.

Results: Among seventy (70) *Staphylococcus aureus* isolated from a variety of specimens, 18 (25.7%) were found to be MRSA, and the remaining were MSSA isolates. Of 18 (25.7%) of ICR *S. aureus*; 13 (72.2%) and 5 (27.8%) were found to be inducible clindamycin resistant *S. aureus* from MRSA and MSSA isolates respectively. The overall prevalence of ICR was found to be 18 (25.7%) and 33 (47%) of isolates were found to be MDR. Prevalence of ICR was found to be 16 (48.5%) in MDR isolates. The association between ICR with MRSA and MDR isolates found to be significant (p=<0.05).

Conclusion: The prevalence of inducible clindamycin resistance (ICR) phenotype was found to be increasing in the western part of Nepal. Increasing ICR emphasizes the need for D-test should be performed routinely in clinical laboratories to avoid treatment failure.

*Keywords***:** Inducible clindamycin resistance (ICR), MRSA, D-test, MDR, western Nepal

Poster Presentation 21

MDR and ESBL *Escherichia coli* in wastewater of Biratnagar in Eastern Nepal

Shrawan Sah[1*], Dhiren Subba Limbu[1], Santosh Thapa[1,2]
[1]*Department of Microbiology, Central Campus of Technology, Tribhuvan University, Dharan, Nepal*
[2]*Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas, USA*
Correspondence: shrawan.sah.54@gmail.com

Background: Sewage contains a wide range of microorganisms, including bacteria, fungi, and protozoa. Bacteria such as *Shigella*, *E. coli*, *Klebsiella*, *Vibrio*, and *Salmonella* can be found in sewage drain water. Hospital sewage often contains various multi-drug resistant bacteria and substances, including antimicrobials, pharmaceuticals, disinfectants, heavy metals, radioisotopes, and drugs that are not metabolized by patients. The presence of pathogenic *E. coli* in the environment of water bodies poses a significant public health concern due to the increased risk of waterborne illnesses.

Methods: During June 2023 to July 2023, a total 9 points of site within the wastewater treatment plant of Biratnagar area and 10 manholes in connected network was selected for sampling. The physio chemical properties of the sewage samples were analysed by APHA method. *E. coli* was isolated using standard method and antibiotic test were performed by double disc method.

Results: Total number of colonies isolated in the sewage samples- influent average of 30 days is $9\times10^{\wedge 6}$ CFU/100 mL (17.06%) with p- value (0.458>0.05) whereas effluent releases in surface water after facultative treatment in average 0.5×10^6 CFU/100 mL (0.94%) with p- value (0.250>0.05). Out of the 117 *E. coli* isolates, 76 (64.95%) were multidrug resistant with p-value (0.457)>0.05. The result was positive towards ESBL by 52.2 % of *E. coli* which was shown by both cephalosporin associated with clavulanic acid with p-value (0.451) >0.05.

Conclusions: There was a high enumeration of *E. coli* in untreated wastewater following through the sewage network. Even after treatment, 10^3 CFU bacteria was released in the effluent. The bacteria surviving the treatment system were resistance to different types of antibiotics. There is a high risk of spreading infectious pathogens in aquatic systems due to the presence of antibiotic resistant bacteria.

Poster Presentation 22

A Typhus and Typhoid fever outbreak: A diagnostic dilemma─ Joypurhat, North-Western part of Bangladesh, 2021

Sohel Rahman[1], Alden Henderson [2], Murshida Khanom[1], Ishrat Siddiqui[3], Tahmina Shirin[1]
[1]Department of Epidemiology, Institute of Epidemiology Disease Control and Research, Dhaka, Bangladesh
[2]Centre for Disease Control, Atlanta, US
[3]Institute of Infectious Diseases, Dhaka, Bangladesh

Background: Typhoid (enteric) and typhus (*Rickettsia*) fevers are endemic in Bangladesh and have similar clinical sign-symptoms but have different treatment and control measures. However, the *Rickettsia felis*, a sub-variety of typhus, is emerging in Bangladesh especially in North-western part of Bangladesh due to vector availability. This study focuses on confirming an outbreak due to concomitant typhoid and typhus fever and reporting an increased incidence of rickettsia fever by less common sub-type of typhus.

Methods: We investigated an outbreak following steps of Outbreak Investigation. We defined cases, searched cases, line listed them and finally described them by epi-curve. Epi-curve study embarked on further evaluation which ended up with concomitant typhus and typhoid fever.

Results: Of 241 cases, 158 (65.6%) cases with typhoid fever, 83 (34.4%) with typhus and 39 (16.2%) with both diseases. But initially outbreak was thought to be enteric fever due to presence of Salmonella typhi in blood culture report. Nevertheless, one physician observed that a typhoid case did not respond to ceftriaxone but was sensitive in blood culture. Moreover, pneumonitis in few cases (complication of typhus) with significant Weil-Felix-Test and treatment response to doxycycline eventually confirmed typhus. However, in this outbreak, the proportion of rickettsia subgroups was Typhus Group (TG) equal to Spotted Group Rickettsia Fever (SGRF) which was greater than the Scrub (STG) Typhus Group (TG=SGRF>STG). This proportion differs from the typical subgroup pattern in Bangladesh of TG>STG>SGRF.

Conclusion: Findings of this outbreak are in-line-with the recent scientific knowledge of outbreak occurrence by two diseases simultaneously and increasing prevalence of the *Rickettsia felis*, a variety of SGRF, in North-western part of Bangladesh. However, physicians must be aware of the importance of distinguishing between typhoid fever and typhus, and epidemiologists must be aware that simultaneous outbreaks of typhoid and typhus fever can occur in the same population at the same time.

Poster Presentation 23

Exploring *Bacillus* species to combat Aflatoxigenic *Aspergillus flavus*: A potential tool for bioremediation

Suprina Sharma[1]*, Dev Raj Joshi[1], Tika Bahadur Karki [2], Reshma Tuladhar[1]
[1] *Central Dept. of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal*
[2] *Central Department of Biotechnology, Kathmandu University, Dhulikhel, Nepal*
**Correspondence: meetsuprina@gmail.com*

Background: Life threating liver cancer due to consumption of aflatoxin contaminated food has been reported in Nepal. *Aspergillus flavus* infested cereal and grain products are the major source for the fungal aflatoxin. We aim to investigate a sustainable biological control strategy for the remedy against toxigenic *A. flavus* by utilizing indigenous potent *Bacillus* spp isolated from soil.

Methods: A total of 50 samples (40 soil samples, 5 compost and 5 food samples) were randomly collected from different places of Nepal. *Bacillus* species were selectively isolated by heating the samples at 80ºC for 10 minutes. Isolation and identification were done by standard microbiological techniques including colony characteristics, Gram's staining, spore staining and biochemical tests. Growth inhibition activity of *A. flavus* by isolated *Bacillus* species was done by dual-culture technique on potato dextrose agar plates. Isolates showing high growth inhibition against *A. flavus* were further identified by 16S rRNA gene sequencing.

Results: Altogether 51 isolates were presumptively identified as *Bacillus* species. Five isolates belonging to *Bacillus subtilis, B. tequilensis, B. licheniformis, B. cereus, B. weidmannii* effectively inhibited the growth of aflatoxigenic *A. flavus* based on 16S rRNA gene sequencing.

Conclusion: This potent *Bacillus* strains showing the antifungal activity against aflatoxigenic *Aspergillus flavus* was identified. This will have implication in commercial production of bioactive anti-aflatoxigenic compound to minimize the postharvest loss in the field of agriculture.

Keywords: Aflatoxin, *Aspergillus flavus, Bacillus* species

Poster Presentation 24

ESBL and MBL producing gram negative bacteria isolated from a patient suspected of urinary tract infection visiting tertiary care hospital

Gajendra Kumar Das
Department of Microbiology, Tri-Chandra Multiple Campus, Institute of Science and Technology,
Tribhuvan University, Kathmandu, Nepal
Correspondence: gajandradas2015@gmail.com

Antibiotic-resistant organisms are associated with higher rates of morbidity and mortality, as well as an increased cost of hospitalization. The most frequent drug resistance mechanism among these often-found bacteria in clinical samples is Beta-lactamase production. The research entitled "ESBL and MBL producing gram negative bacteria isolated from patients suspected of urinary tract infection visiting tertiary care hospital" was conducted from January 2022 to June 2022 at Shukraraj Tropical and Infectious Diseases Hospital, Kathmandu. A total of 714 urine samples were collected from suspected UTI patients and cultured. The isolates were identified as *E. coli*, *Klebsiella* spp and other bacteria using standard biochemical tests and antimicrobial susceptibility pattern was determined using the Kirby-Bauer's disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines. Confirmation of the ESBL producers were done by performing the combined disc test using Cefotaxime and Ceftazidime along with clavulanic acid. Imipenem and meropenem were used in combination with EDTA for MBL detection. Out of 714 urine samples from suspected UTI patients, 145 were found to have growth. Among them 103 were from female and 42 were from male. Similarly, 134 were from outpatients and 11 were from inpatients. The prevalence rate of UTI was the highest 30 in age group 51-60 years followed by 25 from 31-40 years age group. *E. coli* (57.24%) *was* found to be the most predominant isolate followed by *Klebsiella* spp (31.03%), *Pseudomonans aeruginosa* (4.83%), *Citrobacter* spp were (2.76%), *Proteus mirabilis* (2.07%) and *Enterobacter* spp. were (1.38%) and *Enterococcus fecalis* (0.69%). Out of 145 isolates, 111 were MDR strains. ESBL and MBL producers were found 40% and 15.2%, respectively, of the isolates. Colistin (92.8%) and Nitrofurantoin (92.8%) were found to be the most effective antibiotic while Amoxyclav was least effective.

Keywords: Antimicrobial susceptibility pattern, ESBL, MBL, MDR, Uropathogens.

Poster Presentation 25

Candida albicans carrier in oral cavity of patients with diabetes mellitus

Raksha Rimal
Department of Medical Laboratory Technology (B.Sc.MLT),
Nobel College, Affiliated to Pokhara University, Kathmandu, Nepal

Background: Diabetes mellitus (DM) is an ancient metabolic and degenerative disorder. It is characterized by chronic hyperglycemia and causes long term chronic macro vascular and micro vascular complications like retinopathy, neuropathy, and nephropathy. It is acknowledged that the link between Diabetes mellitus and the carriage of *Candida albicans* are more susceptible to fungal infection to Diabetic patients as compared to the healthy group. It may occur due to poor glycemic control, poor hygiene, immune suppression, etc. The main aim of our study was to determine the *C. albicans* carrier in oral cavity of diabetes patients.

Methods: This study included 72 patients, of which there were 36 patients with DM and 36 patients without diabetic mellitus. A total of 10ml of sterile normal saline was used to collect oral rinse sample and centrifuged at 3000 rpm for five minutes. This sample was plated onto SDA with chloramphenicol medium plates and incubated at room temperature for 3-4 days. The plates were visualized daily at 24 hours, 72 hours and followed up to seven days to check for growth. *C. albicans* was found by performing germ tube tests. Yeats corrected Chi-square test and students t-test were used.

Results: The growth *of C. albicans* was found to be statistically significant difference between medicated and non-medicated diabetic patients. Where, patients with medication were highly significant in comparison to non-medicated patients ($\chi2$ (2) = 10.7462; P = 0.004). *C. albicans* was found only in DM patients (6.9%). The mean *C. albicans* was found higher in female (5.52%) than male (1.38%).

Conclusion: A statistically significant difference was found in medicated and non-medicated patients with DM. Our findings indicated that the frequencies of *C. albicans* were seen as a risk in DM patients having a diverse duration of control diabetes.

Poster Presentation 26

DFT calculations for electronic and optical properties of wurtzite Zno structure and molecular docking supported antimicrobial activity

Mohan Bahadur Kshetri, Kamal Khanal, Madhav Prasad Ghimire, Tika Ram Lamichhane*

Central Department of Physics, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: tika.lamichhane@cdp.tu.edu.np*

Introduction: ZnO-based structures are promising candidates for various applications due to their unique properties. This research aims to investigate the electronic properties of ZnO wurtzite structures and to identify the antimicrobial properties of the phytocompound-assisted nanocage.

Methods: The structural and physical properties of the ZnO crystals have been determined by density functional theory (DFT). Through comprehensive DFT calculations with FPLO package, we analyze the changes in the band structure, density of states, and charge distributions. The phytocompound adsorbed ZnO nanocage is optimized using Gaussian 16. The protocols of molecular docking are used to identify the binding free energy, inhibition constant and interactions between ZnO nanocage and active site of microbial protein target.

Results: The band structure and density of states calculation show that wurtzite ZnO behaves has a band gap of 1.0 eV in generalized gradient approximation (GGA). The caffeic acid adsorbed ZnO nanocage shows strong interactions with dengue type 2 virus non-structural protein NS1 resulting in binding free energy of -5.25 kcal/mol and inhibition constant of 0.14 mM.

Conclusion: The ZnO wurtzite structure shows the semiconducting nature with a direct band gap. The caffeic acid adsorbed ZnO nanocage stands as a promising nanomedicine having strong antimicrobial efficacy. It effectively inhibits the dengue type 2 virus non-structural protein NS1. However, further investigations are needed to validate the findings of the computations.

Keywords: DFT, wurtzite ZnO, caffeic acid and ZnO nanocage, molecular docking, binding free energy

Poster Presentation 27

TiO_2 Electronic and Optical Properties and Interactions with Human Serum Albumin by Density Functional Theory and Molecular Docking Approach

Kamal Khanal, Mohan Bahadur Kshetri, Madhav Prasad Ghimire, Tika Ram Lamichhane*

Central Department of Physics, Tribhuvan University, Kirtipur, Kathmandu, Nepal
**Correspondence: tika.lamichhane@cdp.tu.edu.np*

Background: Titanium dioxide (TiO_2)-based materials are found to be promising candidates for various applications due to their unique properties, such as spintronics, photocatalysis, biological and chemical stability. Anatase TiO_2 is a tetragonal structure in space group14_1/amd with lattice constants a=3.782Å and c=9.502Å.

Methods: The structural, electronic and optical properties of TiO_2 materials have been determined by using density functional theory (DFT). In DFT calculations using full potential local orbital (FPLO) software, analyse the changes in band structure, density of states and charge distributions. Zr-doped TiO_2 nanoparticle appears to be an effective inhibitor of human serum albumin (HSA) as identified by molecular docking analysis.

Results: The density of states and band structure calculation show that TiO_2 behaves as a semiconductor with an energy gap of 2.01eV in generalized gradient approximation (GGA). As for optical dielectric functions both real and imaginary parts as functions of photon energy. The Zr-doped TiO_2 nanoparticle in complex with HSA has the binding score of -4.0 kcal/mol with inhibition constant of 1.17 mM showing strong H-bonding interactions.

Conclusion: The anatase TiO_2 behaves as a semiconducting nature with indirect band gaps. Selected transition metal doped as well as undoped TiO_2 nanoparticles can be used for anticancer and antimicrobial activities. The lower the inhibition constant for a particular drug at a particular receptor, the stronger its binding affinity for that receptor.

Keywords: TiO_2 band structure, density functional theory, electronic properties, optical properties, human serum albumin, inhibition.

Poster Presentation 28

Chemical Profiling and Anti-tick and Flea activity of Tar of *Cedrus deodara* from Karnali Region of Nepal

Ram Prasad Baral[3,] *Pramodh* Satyal[1], Rameshwar Adhikari[2] Achyut Adhikari[3,*]
[3] Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[1]Aromatic Plant Research Center, Lehi, UT 84043, USA
[2]RECAST, Tribhuvan University, Kirtipur, Kathmandu, Nepal
E-mail: rpb984950859990@gmail.com

Background: Synthetic pesticides are a huge problem for human beings and both terrestrial and water ecosystems. Pesticide residue in food, vegetables, and water is a cause of many diseases including cancer and heart disease. There is a need for a less toxic biopesticide to save our ecosystem. Cedarwood Deodar, native to the Western Himalayas, is valued for its wood and aromatic essential oil and is renowned in natural medicine for its potent antibacterial properties. Local people use the tar of this plant against ticks and fleas of cattle.

Methods: The tar of the wood of the plant was collected using the traditional pyrolysis method. GC-MS technique was used to identify chemical compounds, Fumigation, repellent and Contact.

Results: Chemical composition, bio-defines activities, deodar 100% tick and flea were killed by 2000ppm within 3 hours.

Conclusions: Tar of Cedrus deodara exhibits diverse biological activities including potential anti -tick and anti-flea characteristics.

4th International Conference on Infectious Diseases and Nanomedicine - 2023 (ICIDN – 2023)

December 15-18, 2023: Kathmandu, Nepal (in-person+online)

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Supported by

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